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STUDIES ON FILM-FORMING YEASTS

II. FILM-FORMING YEASTS IN RENNET BRINE[1]

BY V. E. GRAHAM[2] AND E. G. HASTINGS[3]

Abstract

In the commercial production of rennet extract, calves' stomachs are soaked in brine tanks held at a low temperature. Unless special precautions are taken, a heavy scum forms on these tanks. Salt tolerant yeasts of the genus *Debaryomyces*, which grow well at low temperatures, are chiefly responsible for this scum from which *D. tyrocola*, originally isolated from cheese, and *D. Guilliermondi*, originally isolated from sausages, were isolated. Attempts to isolate these species from the contents of a calf's stomach, salted calves' stomachs, dried calves' stomachs, and soil were unsuccessful. These species did not grow in a medium containing 20% sodium chloride, nor in one in which the pH had been lowered to 2.0.

Introduction

Commercial rennet extract is made by removing rennin from the fourth stomach of a young calf, the extraction being accomplished by prolonged soaking in a brine solution. Some protein and other constituents are removed from the stomachs in this process and the resulting mixture would undoubtedly spoil quickly were it not for the preservative action of the salt and the low temperature maintained in the extracting rooms. If the material in the extracting vats is allowed to remain at rest a grey scum frequently forms on the surface. Vigorous agitation suppresses scum formation. The nature and significance of this scum has not been understood but its presence has been a matter of some concern.

Yeasts Isolated from Rennet Brine

Several samples of scum were obtained and studied by the methods already described (4). Microscopic observation showed that small, round to oval yeasts were present. Inoculation of enrichment medium No. 1 with this material resulted in the formation of a typical scum when the medium was incubated at room temperature. Six strains of film-forming yeasts were isolated from the enrichment cultures by plating on acid glucose yeast extract agar. The pH of this medium was adjusted to 4.5 with lactic acid.

All these cultures, designated R_1, R_2, R_3, R_4, R_{19}, and R_{22}, were found to belong to the genus *Debaryomyces* Klocker. They have been described

[1] *Manuscript received in original form June 2, 1941, and as revised, October 20, 1941.*

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in detail elsewhere (3). Stelling-Dekker (9) characterized the genus as follows: "The cells are predominantly round but may also be oval, and are usually small. Vegetative reproduction takes place by multilateral budding. In wort a sediment is formed, usually also a ring, and many species also form a film. Asci are produced after isogamic or heterogamic copulation or parthenogenically. The spores are round, mostly with an oil drop in the centre. The spore wall is warty, but this is often difficult to observe. In nearly all cases there is only one spore per ascus. Usually there is no fermentation; however, one species has a good fermentation. Nitrate assimilation is negative. On a medium containing ethyl alcohol there is good growth often with the formation of a thin film." The cultures examined in the present study agree with this description except that the results on ethyl alcohol utilization were inconclusive.

Regarding the difficulty in observing projections on the spore wall in this genus, Mrak and Bonar (7) have shown that the ease with which these may be seen is determined chiefly by the size of the spore in relation to the size of the ascus. When cultures are grown at temperatures of 22° C. or higher there is a tendency for the spore to fill the ascus, but when incubated at considerably lower temperatures the spore is smaller in relation to the size of the ascus and the markings are more distinct. In the study reported here, experience with cultures grown at room temperature and in the refrigerator confirms this observation.

Cultures R_1, R_3, and R_4, were identified as *Debaryomyces tyrocola* Konokotina. This species was isolated by Konokotina (5) from "Hollandish" cheese. It is interesting that this organism has now been found in rennet brine. Konokotina isolated four strains of which Stelling-Dekker has described three. The differentiation of the strains is based chiefly on the size of the cells. The spherical cells of Cultures R_1, R_3, and R_4, varied in diameter from 2.4 to 3.5 μ and therefore belong to Strain III. These three cultures formed only light films and are probably only partly responsible for the scum found on the rennet extraction brine.

Cultures R_2, R_{19}, and R_{22}, are probably strains of *Debaryomyces Guilliermondi* Dekker. Stelling-Dekker described two strains of this species, viz., *Fm* and *K*. The cultures referred to correspond closely to her description of Strain *K*. According to Stelling-Dekker this strain liquefies gelatine, sporulates freely on Gorodkowa agar, produces a white, dry, wrinkled film on wort and a "brown–yellow–grey" colour on old wort agar slopes. The development of a pink to light brown colour, particularly when grown at 12° C., was quite marked and differentiated these cultures from all others. This colour was most pronounced on wort agar. It did not develop until the cultures had been incubated for two to three weeks. Cultures R_{19} and R_{22} are almost identical. Culture R_2 is different in some respects and, while it is tentatively classified as a strain of *D. Guilliermondi*, further study may show that it is a new species of this genus. The film formed by these three cultures is white,

with a dry 'mealy' appearance. Undoubtedly this species is responsible for most of the scum on rennet extraction brine.

Both species of *Debaryomyces* isolated in this study grew well in a suitable liquid medium containing 15% of sodium chloride but a 20% solution inhibited growth completely. They did not grow at 37° C. but grew luxuriantly at 12° and 24° C. It is apparent, therefore, that if other conditions are satisfactory, they would develop readily at the temperature and salt concentration used in rennet extraction. Mrak and Bonar (8) reported the isolation from pickle brine of strains of *D. membranaefaciens* Naganishi and *D. Guilliermondi* which grew in a 24% solution of sodium chloride.

Influence of Hydrogen Ion Concentration on Growth of Strains of *Debaryomyces*

A medium containing 0.5% peptone, 1% sodium chloride, and 1% glucose was prepared. The pH of different lots of this medium was altered by the addition of hydrochloric acid or sodium hydroxide. The pH of each lot was determined colorimetrically after sterilization. These media were inoculated with the six strains of *Debaryomyces* isolated from rennet brine and also with the four stock cultures recorded in the table. The relative amount of growth after 10 days' incubation at room temperature is indicated in Table I by the

TABLE I

INFLUENCE OF pH ON GROWTH OF STRAINS OF *Debaryomyces*

Culture	pH of medium					
	2.0	2.5	3.8	4.8	6.8	8.0
R_1	Nil	Nil	++	++	++	+
R_2	Nil	Nil	++	++	++	+
R_3	Nil	±	++	++	++	±
R_4	Nil	±	++	++	++	±
R_{19}	Nil	Nil	+++	+++	+++	+++
R_{22}	Nil	±	+++	+++	+++	+++
D. membranaefaciens	Nil	±	++	++	++	±
D. membranaefaciens var. *hollandicus*	Nil	+	++	++	++	++
D. Guilliermondi	Nil	Nil	++	++	++	++
D. Guilliermondi var. *Nova Zealandicus*	Nil	Nil	+	++	++	++

NOTE: (+++) = *Vigorous growth.*
(++) = *Good growth.*
(+) = *Growth light but definite.*
(±) = *Growth doubtful (islands only).*

conventional symbols. These results show that the growth of all strains was inhibited at a pH of 2.0. At pH 2.5 five strains were completely inhibited and four others developed only small islands of growth. *D. membranaefaciens* var. *hollandicus* Lodder grew slowly at pH 2.5 but this species was not found in the samples of rennet brine examined.

Liquefaction of Gelatine

Stelling-Dekker reported that *D. Guilliermondi* liquefied gelatine in 60 days. Culture R_2, when freshly isolated, liquefied gelatine in 45 days, but when tested a year later it did not cause liquefaction in nine weeks. Cultures R_{19} and R_{22} did not liquefy gelatine in nine weeks. Likewise, stock cultures of *D. Guilliermondi* and *D. Guilliermondi* var. *Nova Zealandicus* Lodder* did not liquefy gelatine in nine weeks. It is doubtful whether liquefaction, when long delayed, is of importance in classifying these species of *Debaryomyces*.

The Source of Film-Forming Yeasts in Rennet

The species of *Debaryomyces* found in rennet extraction brine did not grow at 37° C., and, therefore, one would not expect to find them as natural inhabitants of a calf's stomach. The careful examination of the contents of the third and fourth stomachs of a young calf, by the enrichment procedure, revealed no *Debaryomyces* whatever. Likewise, the examination of salted calves' stomachs and dried (blown) calves' stomachs purchased by a rennet manufacturer revealed no yeasts of this genus. Samples of soil taken to a depth of 2 in. from lawn sod, from a vegetable garden, and from the shore of Lake Mendota, Wis., were also examined but yielded no *Debaryomyces*. Both of the species found in rennet extracting brine were isolated by Césari and Guilliermond (2) from sausages in which these species were responsible for white patches on the casings. They reported that several species of *Debaryomyces* were present on salted meats but that they were not present in salt itself. Mrak and Bonar (8) found strains of *Debaryomyces membranaefaciens* in pickle brine.

A strain of *Debaryomyces tyroloca* Linn. was isolated from the larvae of *Lymantria monacha* Linn. by Batschinsky (1). Kouokotina [*sic*] and Krassihukow (6) reported the isolation of yeasts closely resembling *Debaryomyces tyroloca* from Turkestan grapes, the pericarp of cucumbers, birch sap, beef hides, the larvae of *Agrotis segetum* Schiff., the intestine of a porpoise, the excrement of a fox, a honey comb, and rancid butter. It is evident from these reports that species of *Debaryomyces* may be found on many different materials but the natural source of the contamination of rennet brine is still unknown.

* *Kindly supplied by Dr. E. M. Mrak, Fruit Products Laboratory, University of California, Berkeley, Calif., U.S.A.*

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PEAT AND COMMERCIAL FERTILIZERS AS AMENDMENTS FOR GRAY WOODED SOILS[1]

By R. E. Carlyle[2] and J. D. Newton[3]

Abstract

The nitrates in pot cultures of gray wooded soil were generally affected by the various amendments as follows: increased by applications of neutral or acid subsurface peat and not depressed by neutral or acid surface peat; increased by ammonium phosphate alone and in combination with the surface and subsurface peats, and by ammonium sulphate in combination with the subsurface peats, but slightly depressed by triple superphosphate in combination with the subsurface peats.

The wheat crop yields in pot cultures of gray wooded soil were generally affected by the various amendments as follows: increased somewhat by peat alone, and especially by subsurface peat; increased substantially by ammonium phosphate alone, but increased to a greater extent in all three series by a combination of ammonium phosphate and subsurface peat; increased substantially by ammonium sulphate and subsurface peat, but not by triple superphosphate and peat.

The plate counts of bacteria in treated cultures of gray wooded soil increased for two to four months, and then decreased. The ammonium phosphate plus subsurface peat treatment gave the highest counts, the ammonium phosphate plus surface peat the second highest, and the ammonium sulphate plus subsurface peat the third highest. The differences between the effects of triple superphosphate plus subsurface peat, ammonium phosphate, surface peat, and subsurface peat, were small. The control gave only a small increase during the course of the experiment.

The ratio method showed that there are very large numbers of bacteria in gray wooded soil. Instead of ranging from about 500,000 to about 8,000,000 per gm. of soil as with the plate count method, the numbers varied from about 200,000,000 to 2,000,000,000. The numbers reached a maximum after three months' incubation. The ammonium phosphate plus subsurface peat treatment again gave the highest average number, and the succeeding order was also about the same as in the case of the plate counts.

When mixed with gray wooded soil, surface peat, though highly carbonaceous, did not cause any significant decrease in nitrate at first, and produced some increase later. Straw caused some decrease at first, but the later recovery counterbalanced this loss. Cotton caused a decrease to practically none for about three months, and even when combined with ammonium phosphate caused some decrease. Ammonium phosphate alone and in combination with straw and surface peat produced large increases in nitrate. In a duplicate experiment with black soil the trends were similar, but the nitrate was not reduced to the same extent by straw and cotton, and when ammonium phosphate was added with the organic matter, nitrate was not reduced by peat, straw, or cotton.

Introduction

The gray wooded soil zone of central-western and northern Alberta is the largest of the main soil zones of this province. Gray wooded soils are commonly badly leached, light in colour, poor in fertility, and slightly acid in reaction. They especially lack organic matter and often bake badly when dried. When plowed the field has a light coloured, ashy appearance. These soils are also very deficient in nitrogen and sulphur and somewhat deficient in phosphorus

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and potassium. Consequently they commonly do not produce satisfactory crops, and, as the gray wooded soil districts are gradually being settled, the problem of applying fertilizers to increase yields is becoming very important.

A number of reports of investigations concerning Alberta's gray wooded soils have been published (9, 16, 17, 18, 19, 20). These soils are podsolic in nature, but differ to some extent from the podsolic soils of Eastern Canada and the United States. They are less acid and commonly contain a lime layer in the *B* horizon below the leached layer.

Interspersed among these gray wooded soils are many large and small areas of peat or "muskeg" swamps. These peats are commonly classified as "high moor" or "moss" peats and it has been estimated that they cover possibly as much as 25,000,000 acres in Alberta. The vegetation of Alberta peats has been studied and described by members of the Department of Botany of the University of Alberta (6). The surface layer of these "muskegs" is commonly light in colour, and but slightly decomposed. The underlying layers are darker in colour and decomposed to a greater degree. Analyses of Alberta peats have shown that they usually contain high percentages of cellulose in the surface layers; they are quite variable in nitrogen and phosphorus content, commonly low in potassium and high in sulphur (8, 10, 15, 16).

Experiments with peat as an amendment for gray wooded soil were suggested by the fact that these soils are often very deficient in organic matter, and also by the fact that commercial fertilizers had proved highly beneficial to wheat when applied after clover had been plowed down, in experiments at Breton, Alberta (18). The experiments are particularly apt because in many parts of the gray wooded soil zone the peat swamps are so interspersed that nearly every farmer is within a few miles of such a swamp if he does not actually have one on his own farm. Consequently this investigation was started with the purpose of finding out whether the application of such peats would benefit the gray soils. It was felt that the benefits could be both physical and chemical, but it is mainly with the chemical effects that this investigation is concerned.

Methods

The investigation was carried out mainly with pot and tumbler soil cultures and, in general, the results are based on three determinations. First, wheat crops were grown in pots and yields taken as an indication of whether the different treatments of the gray wooded soil are beneficial or otherwise. Secondly, nitrates in pot cultures were determined at regular intervals as an indication of the rate at which the peat and nitrogenous fertilizer incorporated with the soil are nitrified or made available to plants. Thirdly, the effect of peat and commercial fertilizers on microbiological activity in tumbler cultures of gray wooded soil was measured by making bacterial counts periodically. Also, a comparison was made of the effect of peat and some other cellulosic materials (straw, cotton), in pot cultures, on nitrification of gray soil.

The experiment for the determination of nitrates was done in conjunction with that for crop yields and was laid out as follows.

Gray wooded soil (surface seven inches) from Fallis was sifted through a coarse sieve, mixed uniformly, and weighed into 1-gal., 6-in. glazed pots. Stony Plain high lime peat was used in two experiments and Chip Lake acid peat in another experiment. The coarsely ground peat was mixed uniformly with the soil at the rate of three tons of dry peat per acre (or per 2,000,000 lb. of soil).

The treatments are shown in Table I. There were four replicate pots of each treatment. Three of these were used to grow wheat crops and the fourth in each case was uncropped and kept for the determination of nitrate nitrogen. Reward wheat was sown at the time of applying fertilizers. The pots were watered with distilled water. As far as possible equal numbers of plants were left to develop in each pot and the number was 16. Shortly before each successive crop was planted the soil was emptied out, mixed, and returned to the same crock. About one to two months' time elapsed as a rule between the time of harvesting the crop and replanting; this is indicated in the yield tables (III, V, and VII).

The yields of wheat were obtained by cutting the crop close to the soil and drying thoroughly before weighing and threshing.

Representative composite samples of soil for nitrate determinations were obtained by taking samples from each pot in five different places to the depth of the pot, with a small auger.

The nitrate determination was carried out by the phenoldisulphonic acid method as modified by Harper (5).

In determining bacterial numbers two methods were used, the cultural plate method and the ratio method of counting as developed by Thornton and Gray (14). In the first method, Thornton's medium (13), designed to repress spreading colonies, was used. This medium contains mannitol and asparagine as sources of organic matter, and the pH was adjusted to 7.4. A few modifications were made in the ratio method of counting. It was found that the drop of liquid containing the soil and indigotin suspension dried too quickly on the slide when left exposed to the atmosphere. This resulted in a very uneven distribution of bacteria and indigotin particles on the slide and made counting under the microscope very difficult. To overcome this difficulty the slides were placed in a receptacle where the atmosphere was very humid and the films dried slowly and uniformly. Also, difficulty was experienced in staining the bacteria with the two stains used by Thornton and Gray. The bacteria were found to be stained in many cases a faint pink instead of bright red. It was found, as suggested by Fred and Waksman (4), that immersing the slides in 40% acetic acid for three minutes prior to staining remedied the difficulty to a considerable extent. Finally 32 fields were counted for each sample instead of the greater number suggested by Thornton and Gray (14).

A short comparison of the two methods of counting bacteria would perhaps not be out of place here. The ratio method was found to have certain

advantages. It proved to be much faster up to the point where the actual counts under the microscope are made. This is a decided advantage in that the slides can be prepared in a very short time and then set away to be counted later, as they keep indefinitely once the film is fixed on the slide, stained, and dried. Also, the amount of equipment needed for this method is much smaller than that required to carry out the plate method of counting. Finally, the numbers determined by the ratio method more closely approximate the total numbers of bacteria present in soil, as the counts are tremendously greater. The ratio method, however, was found to have some disadvantages. Counting was found to be tedious when compared to counting colonies on plates. The indigo particles were found to vary considerably in size and they often clumped, making it difficult to estimate the number in a clump. Also it was often difficult to distinguish between bacteria and soil particles, and when making these counts only the stained particles that could definitely be assumed to be bacteria were included.

Results

Experiment I

Nitrification and wheat yields as affected by applications of high lime Stony Plain peat and commercial fertilizers to Fallis gray wooded soil

The plan and results of this experiment are recorded in Tables I, II, and III. It should be noted that the peat was applied at the beginning of the experiment, and the commercial fertilizer at the time of seeding the first and fourth crops. Five successive crops of wheat were grown.

TABLE I

Plan of experiments on wheat yields and nitrification as affected by applications of peat and commercial fertilizers to Fallis gray wooded soil[1]

Treatment	Composition of commercial fertilizers			Application per acre[2]	
	N, %	S, %	P_2O_5, %	Fertilizer, lb.	Peat, lb.
Control soil					
Soil + surface peat					6000
Soil + subsurface peat					6000
Soil + ammonium phosphate	16	14	20	800	
Soil + surface peat + ammonium phosphate	16	14	20	800	6000
Soil + subsurface peat + ammonium phosphate	16	14	20	800	6000
Soil + subsurface peat + triple superphosphate	—	—	43	400	6000
Soil + subsurface peat + ammonium sulphate	20	24	—	600	6000

[1] *In Experiments I, II, and IV, Stony Plain neutral (high lime) peat was used. In Experiment III Chip Lake acid peat was used.*

[2] *Rate is based on surface area of soil in pot.*

Nitrates

After one month the soil treated with ammonium phosphate, with and without peat, and ammonium sulphate with peat, showed decided increases in nitrate nitrogen, whereas the soil treated with triple superphosphate and peat, and with peat alone, remained as low as the control for several months, as shown in Table II. Another significant fact brought out in Table II is that the soil treated with surface and subsurface peat is in no case much lower in nitrate than the control soil, thus indicating that the peat treatments are at least not harmful in this respect. Actually the soil treated with subsurface peat alone was distinctly higher in nitrate, on the average, than the control soil.

TABLE II

NITRATE NITROGEN IN FALLIS GRAY WOODED SOIL TREATED WITH STONY PLAIN PEAT AND VARIOUS FERTILIZERS. EXPERIMENT I

Date of sampling	Treatment							
	Control	Surface peat	Subsurface peat	Amm. phos.	Surface peat + amm. phos.	Subsurface peat + amm. phos.	Subsurface peat + triple superphos.	Subsurface peat + amm. sul.
	Nitrate nitrogen (p.p.m.)							
Nov. /34	2.8	2.7	3.9	4.0	3.4	5.7	2.9	3.8
Dec. /34	1.5	1.4	0.8	29.6	26.7	21.6	1.1	19.5
Jan. /35	6.9	2.3	5.6	14.3	42.1	20.0	3.6	26.7
Feb. /35	19.5	11.4	19.5	42.1	38.1	25.8	16.6	38.1
Mar. /35	20.8	21.7	21.7	32.3	30.3	26.3	25.0	28.7
April /35	12.2	26.3	20.8	17.8	50.0	20.8	11.2	19.2
May /35	16.0	12.4	13.9	28.7	26.0	18.7	12.6	20.7
June /35	17.5	24.4	21.5	31.1	28.0	28.0	20.0	28.0
July /35	18.4	14.0	34.7	33.7	34.1	28.0	17.3	35.0
Aug. /35	13.2	12.0	22.4	33.3	23.3	24.9	18.3	13.7
Sept. /35	12.3	17.2	62.5	42.0	50.0	47.6	32.7	32.2
Oct. /35	23.8	18.5	37.0	50.0	50.0	43.4	25.0	28.5
Nov. /35	8.9	13.9	32.2	100.0	50.0	48.7	22.2	37.0
Dec. /35	11.1	10.6	37.0	17.9	30.3	25.3	26.3	18.8
Jan. /36	21.7	7.8	12.5	22.7	13.0	18.5	6.0	16.7
Feb. /36	33.3	30.3	25.9	100.0	62.5	32.2	31.2	39.2
Mar. /36	12.3	25.0	55.5	58.8	29.4	20.0	35.5	29.4
Nitrate nitrogen average	14.8	14.8	25.1	38.7	34.5	26.8	18.1	25.6
	Moisture, %							
Moisture average	15.0	17.1	16.9	15.8	18.7	16.6	17.9	19.1

Crop Yields

In Table III, it should be noted that the first, fourth, and fifth crops (Fig. 1) were relatively good, and the second and third relatively poor. The third crop yields were reduced to some extent by insect (aphid) injury.

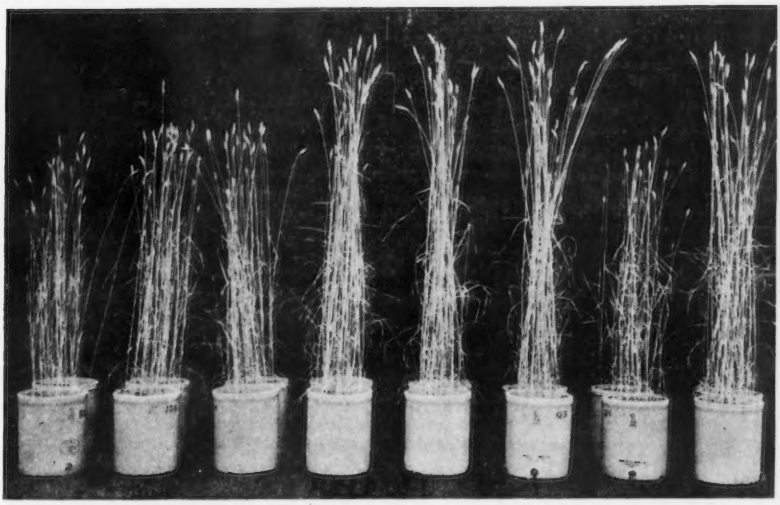

FIG. 1. *Fifth crop of wheat on Fallis gray wooded soil (Experiment I). Only two pots of each treatment were photographed. Treatment: 1. Control; 2. Soil + surface Stony Plain peat; 3. Soil + subsurface Stony Plain peat; 4. Soil + ammonium phosphate; 5. Soil + surface peat + ammonium phosphate; 6. Soil + subsurface peat + ammonium phosphate; 7. Soil + subsurface peat + triple superphosphate; 8. Soil + subsurface peat + ammonium sulphate.*

TABLE III

AVERAGE YIELDS OF FIRST, SECOND, THIRD, FOURTH, AND FIFTH CROPS OF WHEAT IN GRAMS PER POT, FROM FALLIS GRAY WOODED SOIL TREATED WITH STONY PLAIN PEAT AND VARIOUS FERTILIZERS. EXPERIMENT I

Treatment	1st crop[1] Nov. /34 – April /35		2nd crop, May /35 – Aug. /35		3rd crop, Sept. /35 – Feb. /36		4th crop[1] April /36 – Aug. /36		5th crop, Sept. /36 – Feb. /37		Average	
	Grain + straw	Grain	Grain + straw	Grain	Grain + straw	Grain	Grain + straw	Grain	Grain + straw	Grain	Grain + straw	Grain
Control soil	6.4	0.8	6.8	1.5	3.5	1.0	5.9	1.9	5.2	1.5	5.6	1.3
Soil + surface peat	7.1	1.1	5.3	0.6	4.0	1.0	7.1	2.1	5.3	1.6	5.8	1.3
Soil + subsurface peat	8.5	1.9	5.5	0.8	4.8	1.1	8.1	2.2	6.4	1.8	6.7	1.6
Soil + ammonium phosphate	17.8	2.9	7.2	0.9	4.0	0.9	13.4	3.7	12.4	3.3	11.0	2.3
Soil + surface peat + ammonium phosphate	16.0	3.2	7.7	1.1	4.0	0.9	14.5	4.6	12.9	3.8	11.0	2.7
Soil + subsurface peat + ammonium phosphate	19.0	5.2	9.9	3.4	4.4	1.2	16.9	6.0	13.6	4.0	12.8	4.0
Soil + subsurface peat + triple superphosphate	7.2	1.1	4.8	0.6	4.6	1.3	6.7	2.2	6.0	1.7	5.9	1.4
Soil + subsurface peat + ammonium sulphate	12.2	2.6	7.0	0.9	4.2	0.9	15.4	3.6	9.5	2.5	9.7	2.1
Necessary difference at 5% level of significance	2.9	1.2	2.6	1.1	0.9	0.5	1.1	0.7	0.8	0.4		

[1] *Peat applied at time of seeding first crop, and commercial fertilizers at times of seeding first and fourth crops.*

There were no large differences between the control yields and the yields from the surface and subsurface peat treatments, but the peat treatments gave higher average total yields in four out of five crops, and the subsurface peat gave significantly higher total yields in three out of five crops. The subsurface peat treatment in every case gave a higher average yield than the surface. The application of ammonium phosphate gave significantly higher yields than the application of peat alone in the first, fourth, and fifth crops. Ammonium phosphate gave significantly higher yields in combination with subsurface peat than alone in the first, second, fourth, and fifth crops. The pots treated with ammonium sulphate and subsurface peat gave rather smaller yields on the average than those treated with ammonium phosphate alone, and the triple superphosphate with subsurface peat treatment gave little better yields than the controls.

EXPERIMENT II

Nitrification and wheat yields as affected by applications of high lime Stony Plain peat and commercial fertilizers to Fallis gray wooded soil

Experiment II is an exact duplication of Experiment I, except that it was started at a later date. The plan and results of this experiment are recorded in Tables I, IV, and V.

TABLE IV

NITRATE NITROGEN IN FALLIS GRAY WOODED SOIL TREATED WITH STONY PLAIN PEAT AND VARIOUS FERTILIZERS. EXPERIMENT II

Date of sampling	Treatment							
	Control	Surface peat	Subsurface peat	Amm. phos.	Surface peat + amm. phos.	Subsurface peat + amm. phos.	Subsurface peat + triple superphos.	Subsurface peat + amm. sul.
	Nitrate nitrogen (p.p.m.)							
May /35	3.3	4.4	3.3	4.9	4.7	4.9	4.7	5.4
June /35	4.6	7.6	6.4	20.6	5.0	31.1	8.3	22.9
July /35	8.2	17.5	13.6	14.4	17.2	16.9	9.1	30.8
Aug. /35	11.7	6.8	10.2	11.2	10.0	14.2	7.9	3.3
Sept. /35	22.9	25.0	22.2	27.7	11.5	47.6	20.8	13.9
May /36	50.0	41.6	47.6	33.3	33.3	22.2	31.2	40.0
June /36	100.0	37.0	166.6	83.3	34.4	142.0	100.0	76.9
July /36	83.3	90.9	55.5	90.9	50.0	71.4	25.0	43.4
Aug. /36	55.5	47.6	71.4	50.0	52.6	54.0	20.0	55.5
Sept. /36	58.8	83.3	90.9	66.7	55.5	52.6	35.7	47.6
Nitrate nitrogen average	39.8	36.2	48.8	40.3	27.4	45.7	26.3	34.0
	Moisture, %							
Moisture average	18.7	18.9	18.9	17.7	21.3	21.5	19.4	21.5

The pot cultures were maintained from May, 1935, to September, 1936, but nitrates were determined only between May and September each year. Peat and fertilizers were added at the beginning of the experiment only and four crops of wheat were grown in succession.

Nitrates

After one month, as in Experiment I, the gray soil treated with ammonium phosphate and sulphate contained much more nitrate than the untreated, with one exception (Table IV). The exception was the soil treated with surface peat and ammonium phosphate, and in this experiment there is some indication that surface peat tends to depress nitrate accumulation. After two months the nitrate content was not so definitely related to the original applications of ammonium fertilizers. The nitrate content of the soil treated with subsurface peat, on the other hand, was very high in the latter half of this experiment, even greater on the average than that of soil treated with both ammonium phosphate and subsurface peat. The triple superphosphate and subsurface peat treatment gave the lowest average nitrate content in the series, and for this effect no satisfactory explanation is offered.

Crop Yields

The wheat yields of Experiment II are shown in Table V. Significant differences were obtained in the first and third crops, but not in the second and fourth. In the first crop the ammonium phosphate alone, the ammonium

TABLE V

AVERAGE YIELDS OF FIRST, SECOND, THIRD, AND FOURTH CROPS OF WHEAT IN GRAMS PER POT, FROM FALLIS GRAY WOODED SOIL TREATED WITH STONY PLAIN PEAT AND VARIOUS FERTILIZERS.

EXPERIMENT II

Treatment	1st crop[1], May /35 – Aug. /35		2nd crop, Oct. /35 – Feb. /36		3rd crop, April /36 – Aug. /36		4th crop, Aug. /36 – Feb. /37		Average	
	Grain + straw	Grain	Grain + straw	Grain	Grain + straw	Grain	Grain + straw	Grain	Grain + straw	Grain
Control soil	9.2	3.4	3.7	1.1	7.0	2.6	7.7	2.2	6.9	2.3
Soil + surface peat	9.0	3.7	3.5	0.9	7.7	3.0	8.4	2.6	7.1	2.5
Soil + subsurface peat	7.9	3.4	4.0	1.1	8.5	3.3	7.1	2.1	6.9	2.5
Soil + ammonium phosphate	11.8	4.8	3.6	1.0	8.6	3.4	7.1	2.1	7.8	2.8
Soil + surface peat + ammonium phosphate	9.8	5.0	3.7	1.0	8.7	2.9	7.2	1.9	7.3	2.7
Soil + subsurface peat + ammonium phosphate	12.0	4.8	4.3	1.1	11.6	4.5	7.3	2.2	8.8	3.1
Soil + subsurface peat + triple superphosphate	9.2	3.7	3.4	0.8	10.3	4.1	7.1	2.0	7.5	2.6
Soil + subsurface peat + ammonium sulphate	12.3	4.9	3.5	0.8	8.0	2.9	7.6	2.2	7.8	2.7
Necessary difference at 5% level of significance	3.0	1.0	0.9	0.3	1.0	0.8	1.9	0.7		

[1] *Peat and commercial fertilizers applied at time of seeding first crop.*

phosphate with surface and subsurface peat, and the ammonium sulphate with subsurface peat, gave significant increases in yield over the control. In the third crop all of the treatments except surface peat alone gave significant increases in yield. As in Experiment I, the highest yields of the four successive crops on the average were given by the ammonium phosphate with subsurface peat.

EXPERIMENT III

Nitrification and wheat yields as affected by applications of acid Chip Lake peat and commercial fertilizers to Fallis gray wooded soil

Experiment III differs from the two previous experiments in one respect: peat with a distinctly acid reaction was used instead of high lime peat. The plan and results of this experiment are recorded in Tables I, VI, and VII.

TABLE VI

NITRATE NITROGEN IN FALLIS GRAY WOODED SOIL TREATED WITH CHIP LAKE PEAT AND VARIOUS FERTILIZERS. EXPERIMENT III

Date of sampling	Treatment							
	Control	Surface peat	Subsurface peat	Amm. phos.	Surface peat + amm. phos.	Subsurface peat + amm. phos.	Subsurface peat + triple superphos.	Subsurface peat + amm. sul.
	Nitrate nitrogen (p.p.m.)							
June /35	10.8	8.4	9.0	8.4	7.6	7.7	6.7	7.6
July /35	7.0	7.2	11.2	11.9	17.7	11.0	5.8	40.1
Aug. /35	8.3	6.4	6.8	30.3	35.5	32.3	8.1	66.6
Sept. /35	7.8	6.9	19.2	38.1	31.2	28.5	10.2	93.0
Oct. /35	17.5	11.6	13.8	43.4	43.4	41.6	22.2	96.1
Nov. /35	7.8	22.7	12.3	31.2	22.2	80.0	16.7	8.2
Dec. /35	25.0	10.0	15.6	22.7	16.1	15.6	15.2	66.6
Jan. /36	19.2	15.2	13.3	35.7	22.2	31.3	18.9	138.8
Feb. /36	7.1	8.3	7.1	6.5	16.6	10.5	11.9	16.6
Mar. /36	3.9	4.5	3.8	6.3	21.7	3.2	20.0	20.8
April /36*	41.6	45.4	22.7	40.0	47.6	30.3	27.0	55.5
May /36	40.0	41.6	66.6	60.6	58.8	33.3	34.5	71.4
June /36	33.3	17.9	52.6	50.0	111.1	83.3	25.0	100.0
July /36	66.6	76.9	43.4	125.0	—	76.9	32.2	47.6
Aug. /36	56.6	76.9	52.6	125.0	111.1	125.0	50.0	125.0
Sept. /36	20.0	55.5	51.3	90.9	62.5	70.4	37.0	90.9
Nitrate nitrogen average	23.3	26.0	25.1	45.4	41.7	42.6	21.3	58.3
	Moisture, %							
Moisture average	13.7	16.5	15.6	15.3	16.4	16.3	15.6	16.0

** A second application of commercial fertilizer only was made at this time.*

Pot cultures were maintained from June, 1935, to February, 1937. Four successive crops of wheat were grown during this period, but the monthly nitrate determinations were stopped in September, 1936. Peat and fertilizers were added at the beginning of the experiment and a second application of fertilizers only in April, 1936, at the time of seeding the third crop.

Nitrates

In Experiment III (as in Experiment I) there is no evidence that applications of surface or subsurface peat depress nitrification (Table VI). Ammonium phosphate and surface peat produced about the same increase in average nitrate content as ammonium phosphate and subsurface peat but the increase was less than that produced by ammonium phosphate alone. The highest average nitrate was given by ammonium sulphate with subsurface peat. Triple superphosphate and subsurface peat again gave a low average nitrate content, ranking just below that of the control.

Crop Yields

The wheat yields of Experiment III are shown in Table VII. Significant differences were obtained in all four crops, but the yields of the second crop were poor. The crop yields, when averaged, show the same general trends as in Experiments I and II.

TABLE VII

AVERAGE YIELDS OF FIRST, SECOND, THIRD, AND FOURTH CROPS OF WHEAT IN GRAMS PER POT, FROM FALLIS GRAY WOODED SOIL TREATED WITH CHIP LAKE ACID PEAT AND VARIOUS FERTILIZERS. EXPERIMENT III

Treatment	1st crop[1], June /35 – Sept. /35		2nd crop, Oct. /35 – Feb. /36		3rd crop[1], April /36 – Aug. /36		4th crop, Aug. /36 – Feb. /37		Average	
	Grain + straw	Grain	Grain + straw	Grain	Grain + straw	Grain	Grain + straw	Grain	Grain + straw	Grain
Control soil	6.6	2.3	2.2	0.3	8.9	2.9	5.5	1.8	5.8	1.8
Soil + surface peat	8.8	3.2	2.1	0.4	6.4	1.9	7.7	2.5	6.2	2.0
Soil + subsurface peat	11.3	6.7	2.1	0.5	6.6	2.2	6.6	2.2	6.6	2.9
Soil + ammonium phosphate	12.3	6.8	2.9	0.7	12.3	5.3	11.1	3.6	9.6	4.1
Soil + surface peat + ammonium phosphate	16.6	6.8	2.4	0.6	12.0	5.1	11.9	3.8	10.7	4.1
Soil + subsurface peat + ammonium phosphate	18.2	7.8	2.5	0.7	13.1	6.0	10.6	3.3	11.1	4.4
Soil + subsurface peat + triple superphosphate	12.5	4.5	2.7	0.7	6.6	2.5	5.2	1.5	6.7	2.3
Soil + subsurface peat + ammonium sulphate	18.2	6.4	2.8	0.7	8.6	3.4	11.4	3.5	10.2	3.5
Necessary difference at 5% level of significance	4.5	1.3	0.4	0.2	1.2	0.9	1.9	0.7		

[1] *Peat applied at time of seeding first crop, and commercial fertilizers at times of seeding first and third crops.*

The ammonium phosphate alone and in combination with surface and subsurface peat gave significant increases in yield in all four crops, and the ammonium sulphate in combination with subsurface peat gave significant increases in three out of four crops. Treatment with triple superphosphate combined with subsurface peat gave about the same average increase as treatment with subsurface peat alone. Peat alone increased the yields somewhat and, as in Experiment I, subsurface peat gave larger average increases than surface. The ammonium phosphate in combination with subsurface peat gave a significantly higher yield than ammonium phosphate alone in the first crop, and, as in Experiments I and II, the highest average total and grain yields for all crops were given by this combination.

Experiment IV

Effects of applications of high lime Stony Plain peat and commercial fertilizers on bacterial numbers in Fallis gray wooded soil

This experiment was carried out with the object of finding out how treatments of peat and commercial fertilizers would affect the numbers of bacteria in gray wooded soil. As stated in the outline of the investigation, the bacterial numbers were determined by the plate method first; then the experiment was repeated and the numbers were determined by the ratio method.

The results of the counts made by the plate method are shown in Table VIII. The numbers did not increase much at any time in the control soil. In the treated soil the highest counts were obtained after two, three, or four months' incubation, after which time the numbers decreased. The highest average count for the entire period of incubation was given by ammonium phosphate combined with subsurface peat, the second highest by ammonium phosphate with surface peat, and the third highest by ammonium sulphate with subsurface peat. The differences between the average counts given by treatment with ammonium phosphate, surface peat, subsurface peat, and triple superphosphate combined with subsurface peat, were small.

In studying Table IX the first and most striking feature is the great number of bacteria shown by the ratio method. Instead of the bacterial numbers ranging about 500,000 to about 8,000,000 per gm. of soil as shown by the plate count method, the numbers varied as a rule from about 200,000,000 to 2,000,000,000 by the ratio method. Thus it is evident that only a very small fraction of the bacteria actually present in the soil will grow on ordinary nutrient media.

Table IX shows that the bacterial numbers were considerably higher in the soil treated with peat and commercial fertilizers than in the control soil. The soil treated with subsurface peat and ammonium phosphate was the first to show high numbers of bacteria and contained the highest average number of bacteria. The averages show that the surface peat plus ammonium phosphate ranks second and the subsurface peat plus ammonium sulphate third in bacterial numbers. The differences in average counts between the

TABLE VIII

NUMBERS OF BACTERIA DETERMINED BY PLATE COUNT METHOD IN FALLIS GRAY WOODED SOIL TREATED WITH STONY PLAIN PEAT AND VARIOUS FERTILIZERS. EXPERIMENT IV

Date of sampling, 1935	Treatment							
	Control	Surface peat	Sub-surface peat	Amm. phos.	Surface peat + amm. phos.	Sub-surface peat + amm. phos.	Sub-surface peat + triple super-phos.	Sub-surface peat + amm. sul.
	Bacteria (millions per gram)							
May 28	2.1	1.7	1.4	2.2	1.7	1.6	1.7	1.3
June 11	1.8	2.5	2.8	2.2	2.7	3.6	2.3	2.6
June 25	1.7	4.6	4.6	3.3	4.1	7.0	3.3	3.0
July 9	1.6	2.6	1.9	3.2	4.3	4.2	2.7	3.4
Aug. 6	2.2	3.7	4.3	4.3	7.6	5.9	5.1	7.3
Aug. 20	1.0	1.1	1.6	1.3	1.5	1.4	0.8	1.5
Sept. 3	1.1	1.6	0.9	1.3	2.7	1.8	1.1	1.7
Sept. 17	1.5	2.3	2.6	2.6	1.9	2.6	1.8	2.8
Oct. 1	0.6	0.8	0.7	1.3	1.4	1.9	0.9	1.2
Oct. 15	1.7	1.9	2.1	2.2	1.9	1.9	1.8	2.0
Average number	1.5	2.3	2.3	2.4	3.0	3.2	2.1	2.7

TABLE IX

NUMBERS OF BACTERIA, DETERMINED BY DIRECT RATIO METHOD OF COUNTING, IN FALLIS GRAY WOODED SOIL TREATED WITH STONY PLAIN PEAT AND VARIOUS FERTILIZERS. EXPERIMENT IV

Date of sampling, 1936	Treatment							
	Control	Surface peat	Sub-surface peat	Amm. phos.	Surface peat + amm. phos.	Sub-surface peat + amm. phos.	Sub-surface peat + triple super-phos.	Sub-surface peat + amm. sul.
	Bacteria (millions per gram)							
June 24	373	421	545	601	370	343	430	241
July 8	476	971	760	492	627	446	342	499
July 22	315	465	603	763	653	748	462	460
Aug. 6	294	438	575	442	672	1216	412	787
Aug. 20	198	428	433	476	758	756	578	762
Sept. 3	850	1013	1048	1966	1982	2016	1260	1613
Oct. 12	41	329	694	578	1219	1181	1085	1087
Oct. 26	452	610	867	595	826	870	522	803
Nov. 2	223	244	280	238	304	290	289	405
Nov. 16	178	272	317	277	250	254	390	249
Average number	340	519	612	643	766	812	577	691

other treated plots are not significant. The bacterial counts were found to reach a maximum about three months after the application of the peat and fertilizers. From then on the bacterial numbers began to level out until by the end of the experiment, which was carried on for about five months, there were no large differences between the different treatments.

With one unimportant exception the averages given in Tables VIII and IX show that the bacterial numbers as affected by treatment rank in the same order in the ratio as in the plate count method. The same general conclusions regarding effects of amendments will therefore hold.

Experiment V

Effect of peat, straw, and cotton on nitrate nitrogen content of mineral soils

Because a great deal of experimental work has shown that certain kinds of organic matter when added to mineral soil depress the nitrate nitrogen content for a time, and because in this investigation peat was found to have rather the opposite effect, it was felt necessary to set up an experiment where surface Stony Plain peat high in cellulose could be compared with straw and cotton, also high in cellulose. These forms of organic matter were added separately and in combination with ammonium phosphate. Two different types of soil were used, a black fertile soil and a gray wooded relatively infertile soil. The results of the experiment are shown in Tables X and XI.

When mixed with gray wooded soil, surface peat, although high in cellulose did not cause any significant decrease in nitrate accumulation at first although some increase was produced later. Straw caused a decided decrease at first, but a recovery was made after the first month and gains counterbalanced the earlier losses. Cotton reduced the nitrate to practically nothing for a period of about three months. Ammonium phosphate greatly increased nitrate accumulation, and when ammonium phosphate was added together with organic matter, nitrate accumulation was greatly retarded by cotton, but not by straw or peat.

In the case of the black soil the trends are somewhat the same, although the applications of straw and cotton do not appear to be quite so harmful as in the case of the gray soil. The peat did not cause any significant decrease in nitrate accumulation, whereas the straw caused a small decrease at first and the cotton caused considerable decrease (Table XI). Nitrate accumulation was greatly stimulated by the addition of ammonium phosphate alone, and when ammonium phosphate was added together with organic matter, nitrate accumulation apparently was not retarded by the peat, straw, or cotton.

Discussion

A great deal of experimental work on the reclamation of peat has been done in Europe and America, but comparatively few investigations on the direct application of peat to improve mineral soils have been reported. It is interesting to note that the opposite practice, that of applying mineral soil or sand to peat, in order to improve the peat, is well established in Europe.

TABLE X

NITRATE NITROGEN IN FALLIS GRAY WOODED SOIL TREATED WITH STONY PLAIN PEAT, WHEAT STRAW, COTTON, AND AMMONIUM PHOSPHATE FERTILIZER. EXPERIMENT V

Date of sampling	Treatment							
	Control	Surface peat	Amm. phos.	Surface peat + amm. phos.	Straw	Straw + amm. phos.	Cotton	Cotton + amm. phos.
	Nitrate nitrogen (p.p.m.)							
Dec. 4/35	10.0	8.6	8.6	9.1	6.3	4.2	6.0	3.5
Dec. 13/35	8.1	11.9	10.0	21.3	Trace	13.2	Trace	6.8
Jan. /36	6.1	6.9	33.3	47.6	5.1	47.6	Trace	21.7
Feb. /36	15.2	11.4	44.4	43.4	13.9	45.4	0.0	6.7
Mar. /36	9.2	31.4	40.0	47.6	12.8	12.5	7.6	32.7
April /36	33.3	40.0	108.6	89.2	23.8	58.8	12.5	50.0
May /36	25.0	35.7	23.8	54.0	38.5	20.0	12.8	25.0
June /36	39.2	29.4	105.0	31.2	90.9	71.4	45.4	31.2
July /36	166.7	47.6	192.3	312.5	138.9	250.0	78.1	100.0
Aug. /36	90.9	66.6	83.3	111.1	71.4	125.0	35.7	71.4
Sept. /36	71.4	156.3	178.6	208.3	83.3	104.2	62.5	104.2
Nitrate nitrogen average	43.2	40.5	75.3	88.7	44.1	68.4	23.7	41.2
	Moisture, %							
Moisture Average	14.9	15.2	16.0	16.3	16.7	17.3	17.3	18.7

TABLE XI

NITRATE NITROGEN IN EDMONTON BLACK SOIL TREATED WITH STONY PLAIN PEAT, WHEAT STRAW, COTTON, AND AMMONIUM PHOSPHATE FERTILIZER. EXPERIMENT V

Date of sampling	Treatment							
	Control	Surface peat	Amm. phos.	Surface peat + amm. phos.	Straw	Straw + amm. phos.	Cotton	Cotton + amm. phos.
	Nitrate nitrogen (p.p.m.)							
Dec. /35	33.3	50.0	15.6	14.0	22.7	33.3	21.7	23.8
Jan. /36	48.7	51.2	104.0	55.5	40.8	89.2	23.8	89.2
Feb. /36	46.8	37.5	75.0	96.1	30.8	166.6	37.5	62.5
Mar. /36	27.7	22.7	50.0	76.9	45.4	50.0	31.3	119.0
April /36	34.4	27.7	76.9	131.5	35.7	62.5	27.7	40.0
May /36	45.4	55.5	55.5	66.6	66.6	71.4	34.4	62.5
June /36	131.5	108.6	156.2	156.2	125.0	138.8	86.2	131.5
Nitrate nitrogen average	52.5	50.4	76.2	85.3	52.4	87.4	37.5	75.5
	Moisture, %							
Moisture average	32.8	33.6	35.0	36.5	33.8	31.6	30.8	33.4

The investigation reported in this paper was concerned chiefly with chemical, microbiological, and general fertility questions. No attempt was made to cover the physical problems. However, it is felt that if the physical side were studied, the application of peat to gray wooded soils would be found to be decidedly beneficial from this standpoint, because it was noticed in handling the soil that where peat was applied there appeared to be more structure, less tendency to bake, and a greater moisture holding capacity. Dachnowski-Stokes (3) states that the application of poorly decomposed peat to soils, where there is a danger of drought, and where rainfall is light during the summer months, will do much to improve their structure and moisture holding capacity.

The fertilizer treatment plan of the experiment was not made more complete because of the labour involved in carrying out a more complete plan. The one fertilizer that was applied alone, as well as in combination with neutral and acid surface and subsurface peat, was ammonium phosphate 16–20. This fertilizer contains sulphur as well as nitrogen and phosphorus, and is one of the best general purpose fertilizers for gray wooded soils.

These experiments show that from a chemical standpoint the application of such peats as were used is definitely not harmful, but rather beneficial. This is noteworthy because some experimenters have found that other forms of organic matter, with as high a carbon:nitrogen ratio as that of some of the peat used, tend to reduce the nitrate content of the soil. Previous investigations (10) showed that nitrate accumulation occurred in Alberta peat in spite of the peat's low nitrogen content, or high carbon:nitrogen ratio. This may be due to differences in availability of carbon and nitrogen, for, as pointed out by Norman (11), if a portion of the carbon is relatively unavailable the true ratio may be considerably narrower. McCool (7) found that nitrate formation took place in peat and that peat was not inert in this respect. Baur (2) reported that in composting a very acid undecomposed peat nitrate accumulation took place if lime were added, and also that accumulation took place more quickly where a nitrogen–phosphorus–potassium fertilizer was added together with lime.

Scott (12) and other workers state that the application of wheat straw to soil causes a marked decrease in nitrates for a certain period of time. In the experiments reported in this paper it was shown that straw causes some decrease in nitrate when first applied to gray soil, and cotton, a relatively pure form of cellulose, causes a great decrease. Anderson (1) states that nitrification can proceed in the presence of cellulose, but unless nitrogen is present in quantities above that required by the bacteria, the nitrates are utilized as rapidly as they are formed. Other investigators (working with such materials as wheat straw) have found that nitrogen of organic matter in soils can appear as nitrate only if the carbon:nitrogen ratio is 12 or less.

McCool (7) reports that a combination of peat and fertilizer salts proved beneficial to unproductive surface sandy loam soil. Furthermore he states that a satisfactory soil may be made up by using a good grade of peat, commercial fertilizers, and a poor surface soil, sand, or subsoil. The experiments reported in this paper show that wheat crop yields were generally increased somewhat by peat alone (especially by subsurface peat), and increased substantially by ammonium phosphate alone, but that the largest increases in all three series were produced by a combination of ammonium phosphate and subsurface peat.

In previously reported experiments with Alberta peats (8, 10) it was shown that large increases in bacterial numbers (determined by plate counting) were produced by the addition of certain mineral fertilizers to peat soil. Baur (2) found that in composting acid peat the bacterial numbers were increased substantially, and that in completely fertilized and limed samples the numbers were increased still more. The experiments reported in this paper show that the peat alone and the ammonium phosphate alone produced some increase in bacterial numbers, but that the largest increases as determined by both the plate count and ratio methods, were given by treatment with a combination of peat and ammonium phosphate. The numbers of bacteria shown by the ratio method were very great. Instead of ranging from about 500,000 to about 8,000,000 per gm. of soil as in the plate count method, the numbers varied from about 200,000,000 to 2,000,000,000 in the ratio method.

Some field tests of peat and commercial fertilizers, applied separately and in combination, as amendments for gray wooded soil, have been carried on for several seasons, and will be published at a later date.

Acknowledgments

The writers wish to express their appreciation to Dr. F. A. Wyatt, Head of the Department of Soils, for counsel and support of the work, to Dr. J. W. Hopkins of the National Research Council, for advice regarding statistical treatment of yield data, and to the Carnegie Research Grants Committee of the University of Alberta for necessary financial assistance.

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CAROTENE IN FEED GRASSES[1]

By John Allardyce[2] and Douglas Milsom[3]

Abstract

Cuttings of feed grasses less than 7 in. high, taken from the same field and given the same treatment, were found to vary considerably in carotene content. Factors that were found to contribute to these variations were: the amount of sunshine and rain prior to each cutting, the height of the cutting, and the manner of storage. Higher carotene content was found when the cuttings were taken following periods of increased daily sunshine particularly if these periods were preceded by rain. Cuttings less than 7 in. high contained larger amounts of carotene than did 12 in. cuttings. Storing the ground, dehydrated feed grasses in 100-lb. paper-lined burlap sacks at 35° F. during the summer months reduced the average loss in carotene content to 7.5%.

Introduction

The value of alfalfa in live-stock feeds has long been recognized. Today on the Pacific coast of Canada and of the United States the price paid by feed companies for alfalfa and feed grasses in general is based primarily on carotene content. Findings reported by Crabtree (2) indicate that field grasses, grass and clover mixtures, and oat grass are comparable with alfalfa in carotene content. In 1938, Johnson (6) reported the carotene value for green alfalfa as 22.7 mg. per 100 gm. dry matter. This is in keeping with the 15 to 25 mg. values found by Milsom (7) in 1940. More recently Crabtree (2) has confirmed Milsom's findings, and reports greater yields of carotene (35 to 39 mg. per 100 gm.) in both alfalfa and grass crops on which fertilizers were used.

In British Columbia, field grasses are easily obtained and require little cultivation especially if grown on delta land. In the Sumas area they have to some extent begun to replace alfalfa as a source of carotene (2). It has been noted that cuttings taken at different times from the same field may show considerable variation in carotene content and consequently will bring different prices to the farmer.

In the present investigation, these variations have been studied in relation to the natural conditions that occur during production and storage of these feeds. Variables considered as possibly affecting the carotene content include hours of sunshine, temperature range, rainfall, height of grass at cutting, and storage conditions.

Von Hausen (5) in 1933 investigated the effect of varying the nitrogen–potassium–phosphorus ratio on the carotene content and came to the conclusion that both the amount and ratio of these elements were important factors. The ratio producing maximum growth of greenstuff also produced the highest carotene content.

[1] *Manuscript received May 23, 1941, and as revised September 29, 1941.*

Contribution from the Department of Biology and Botany, University of British Columbia, Vancouver, B.C., with financial assistance from Buckerfield's Ltd., Vancouver.

[2] *Assistant Professor of Biology.*

[3] *Student in Fourth Year Arts.*

Fraps and Kemmerer (4) investigated carotene losses in alfalfa and feed mixtures during storage and found these much higher in summer than in winter. In alfalfa placed in open jars at room temperature for eight weeks, carotene losses ranged from 6 to 70%. Under like conditions, at 33° to 35° F., the losses ranged from 0 to 26%.

Fagan and Ashton (3) in 1938 studied various methods of drying grasses and their effects on the carotene content. They found that natural field drying causes losses as high as 66%. This loss was cut approximately in half by the use of mechanical driers. The process consisted in passing the freshly cut grass through a chamber held at temperatures ranging from 1000° to 1600° F. and at speeds that required from two to five minutes. Minimum losses occurred when the drier was held at 1600° F. and the grass run through in two minutes.

Procedure

The grasses used in the investigation were grown on the Vancouver City Airport, delta land on Sea Island at the mouth of the Fraser River. The field grasses are those common to this area and include Yorkshire fog (*Holcus lanatus*), Kentucky blue (*Poa pratensis*), bent (*Agrostis*), and timothy (*Phleum pratense*), together with a small mount of white clover (*Trifolium repens*) and red clover (*Trifolium pratense*).

Cuttings of grass less than 7 in. high were made from June 12 to September 12 from the whole field, except a portion permitted to grow to a height of 12 in. The carotene content of this grass was compared with that of a 7-in. cutting made on the same day from the same field.

All cuttings were dehydrated by heating in a drier at 1400° F. for one minute and were then freely ground in a process requiring two minutes. The grass was then taken to the mill where it was reground and the whole cutting was thoroughly mixed before sacking in 100-lb. paper-lined burlap sacks. Samples drawn during the sacking process were thoroughly mixed and samples were removed for the determination of carotene by the method of Bolin and Khalapur (1). The 100-lb. sacks were stored at 35° F. during the summer months. On July 6, samples for carotene determination were withdrawn from sacks of the ground, dried grasses taken at random. Four subsequent determinations were made on each of the sacks.

Records of weather conditions over the experimental period were obtained from the Dominion Meteorological Station situated in the area under investigation.

The carotene value for each cutting made during the experimental period was plotted against the day of cutting and compared with the average number of hours of sunshine on the day of cutting, on one, two, three, four, and five days previous to cutting, and for the whole interval between consecutive cuttings. The sunshine curve finally used (Fig. 1) was based on the average daily sunshine for the four days previous to cutting and, since the intervals between cuttings were not always the same, the average was determined not

only for the day of cutting but for each day of the experimental period. Hence each point on the sunshine curve represents the average hours of sunshine per day for the previous four days.

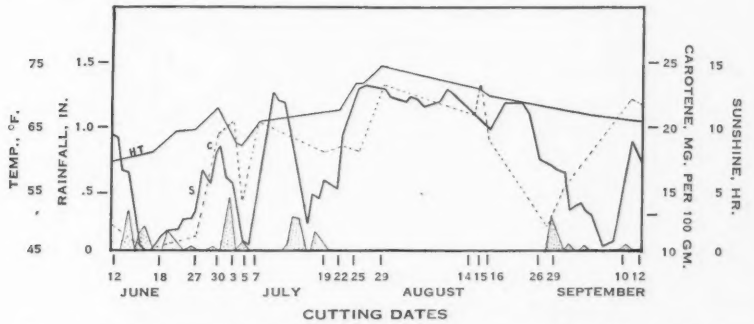

FIG. 1. *C = carotene; S = sunshine; HT = maximum temperature;* ▤ *= rainfall.*

Results and Discussion

In Table I and Fig. 1 appear the carotene values of cuttings made on the dates shown. Analyses were made within three days of each cutting on the ground, dried samples.

TABLE I

CAROTENE LEVELS DURING EXPERIMENTAL PERIOD

Date	Carotene, mg. per 100 gm.	Date	Carotene, mg. per 100 gm.	Date	Carotene, mg. per 100 gm.
June:		July:		August:	
12	12.2	7	20.8	15	23.9
18	10.0	19	18.2	16	19.1
27	11.2	22	18.7	26	12.0
30	19.8	25	18.3	29	15.2
		29	23.9		
July:		August:		September:	
3	20.8			10	22.7
5	14.3	14	21.4	12	22.2

As may be seen from Fig. 1, both the carotene and the sunshine curves begin and end with a peak. Each shows a second peak at the end of June, a third in the first part of July, and a more or less sustained peak from the middle of July to the middle of August. While these trends are not close enough to allow a mathematical correlation, they would seem to indicate that when the average daily sunshine for the four days previous to cutting was high, the carotene content was high.

Other factors, however, are evidently at work. From July 20 to August 26, there was a hot dry period. About the middle of August the carotene curve

began to drop—a week before the sunshine curve. At the bottom of the carotene drop it began to rain. Despite the continued decrease in sunshine over the next 10 days, and rain for a week, the carotene content rose to a new peak with the next sunshine peak. It will thus be noted that rain followed by sunshine, which stimulates plant growth, also stimulated increased production of carotene. Moreover, peaks in the maximum temperature curve appear to coincide with or slightly precede the peaks in the carotene curve.

It is to be noted that since the whole experimental area of any given height was cut on the same day and the cuttings were always given the same treatment in drying and grinding and then were thoroughly mixed before samples were drawn, variations in the mineral distribution over the area or in the distribution of the grasses and the clover found growing there cannot account for the change in the carotene content at different times of cutting.

Carotene determinations made on feed grasses cut at heights of 7 and 12 in. on the same day from the same field were 22.9 and 11.3 mg. per 100 gm., respectively.

TABLE II

CAROTENE CONTENT OF DRIED GRASS AFTER STORAGE AT 35° F., MG. PER 100 GM. DRIED GRASS

Sack	July 6	July 19	Aug. 2	Aug. 23	Sept. 6
1	13.97	13.68	13.54	13.54	13.09
2	16.03	15.89	15.89	15.72	15.15
3	10.00	9.86	9.86	9.86	8.82
4	12.06		11.92	11.92	11.77
5	9.12	9.12	9.12	9.12	9.12
6	8.09	7.94	8.09	8.09	8.09
7	10.30	10.00	9.77	9.50	8.83
8	23.98	22.60	21.62	21.62	19.42

The average loss of carotene from all sacks of stored feed grasses tested was 7.5%. The results for the first eight of these sacks appear in Table II. From a comparison of the average value with the results obtained by Fraps and Kemmerer (4) and Fagan and Ashton (3), it would appear that excessive losses in carotene content of feed grasses during the summer months can be prevented by the grinding and drying process. More recently Crabtree (2) has found that during storage of alfalfa and feed grasses under similar conditions, greater losses of carotene occurred in the alfalfa.

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ZYGOSACCHAROMYCES NECTAROPHILUS N. SP. AND *ZYGOSACCHAROMYCES RUGOSUS* N. SP.[1]

By A. G. Lochhead[2]

Abstract

Two species of osmophilic yeasts, previously described but not named, have been designated respectively *Zygosaccharomyces nectarophilus* Lochhead and Farrell and *Zygosaccharomyces rugosus* Lochhead and Farrell.

Introduction

During the course of previous studies in this laboratory on osmophilic yeasts causing fermentation in solutions of high sugar concentration, various species of *Torulopsis*, *Saccharomyces*, *Schizosaccharomyces*, and *Zygosaccharomyces* were isolated and described. The yeasts studied included previously described species, as well as several new species, two of which were named *Zygosaccharomyces nussbaumeri* Lochhead and Heron and *Zygosaccharomyces richteri* Lochhead and Heron, respectively (3).

Two other species of *Zygosaccharomyces*, isolated from a variety of sources during the investigations, and identified only by number, were believed to be new species not elsewhere described, a view that later studies have confirmed. In the manuscript of an "Annual Report", prepared some years later, these yeasts were accordingly given the names *Zygosaccharomyces nectarophilus* Lochhead and Farrell and *Zygosaccharomyces rugosus* Lochhead and Farrell, and type cultures were sent to the Centraalbureau voor Schimmelcultures at Baarn. In view of the fact that the manuscript referred to was not published, and the names, therefore, have not yet effectively reached the literature, the new species are hereby designated.

Zygosaccharomyces nectarophilus has been described (with illustrations) as cultures *N*4 (3), *S3B*2 (1), and 155*Y* (2), and *Zygosaccharomyces rugosus* as cultures *S3B*11 (1) and 139 (2). To the earlier morphological and physiological descriptions of these yeasts, it is now possible to add further characterization based on recent comparative studies of the nutrilite requirements of osmophilic yeasts (4). For growth of *Z. rugosus*, biotin is the essential growth factor, though development is stimulated by pantothenic acid. Inositol, thiamin, and pyridoxin are non-essential nutrilites. In the case of *Z. nectarophilus* both biotin and pantothenic acid are necessary for multiplication, with inositol an important additional factor in providing optimum growth.

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Contribution No. 140 (Journal Series) from the Division of Bacteriology and Dairy Research, Science Service, Department of Agriculture, Ottawa, Canada.

[2] *Dominion Agricultural Bacteriologist.*

Description of Species

Zygosaccharomyces nectarophilus n. sp. Lochhead and Farrell

In young cultures cells round to slightly oval, generally 3 to 5 μ in diameter, with extremes of 2 to 6 μ. In old cultures "giant" cells may be seen, 9 to 10 μ in diameter. Reproduces asexually by budding, and by isogamic copulation resulting in ascospore formation. The ascus contains one to four spores, usually two to three. Spores round to slightly oval, mostly 3.5 to 4 μ in diameter, with variations between 3 and 4.5 μ. The yeast is able to develop in solutions of high sugar concentration. Produces alcoholic fermentation in dextrose, levulose, and to a lesser degree in mannose. Does not ferment arabinose, xylose, galactose, saccharose, maltose, lactose, raffinose, dextrin, mannitol, dulcitol, or salicin. Giant colony raised with little spreading, irregular in outline with broken surface. Growth scanty on carrot and potato. Induces no change in milk, and does not liquefy gelatine. Has been isolated from floral nectar, honey, and soil. Requires biotin, pantothenic acid, and inositol for normal growth.

Zygosaccharomyces rugosus n. sp. Lochhead and Farrell

In young cultures cells oval to round, the majority 4 to 6 μ in length and 3 to 4 μ wide. Reproduces asexually by budding and by isogamic copulation resulting in ascospore formation. The ascus contains one to four spores, usually two to three. Spores slightly oval to round, the majority 3 to 3.5 μ in length. The yeast is able to develop in solutions of high sugar concentration. Causes alcoholic fermentation of dextrose, levulose, mannose, saccharose, and maltose. Does not ferment arabinose, xylose, galactose, lactose, raffinose, dextrin, mannitol, dulcitol, or salicin. Giant colony round, spreading, with surface becoming covered with coarse folds. Growth abundant on carrot and potato. Causes no change in milk, but liquefies gelatine slowly. Has been isolated from honey and soil. Requires biotin and pantothenic acid for optimum growth; inositol not essential.

Descriptions of the two species in Latin are given herewith.

Zygosaccharomyces nectarophilus sp. nov.

In culturis juvenilibus, cellulis rotundatis vel leviter ovalibus, 3–5 μ (2–6 μ); in culturis senioribus per occasionem cellulis giganteis, 9–10 μ. Regenerat et gemmis et copulatione aequarum cellularum ascosporis deinde orientibus. Ascis 1–4 (plerumque 2–3)-sporis; sporis rotundatis vel leviter ovalibus, 3.5–4 μ (3–4.5 μ). In diluto forti sacchari crescere potest. Inducit fermentationem alcoholicam in dextrosio, levulosio, et in minorem modum in mannosio sed nullam in arabinosio, xylosio, galactosio, saccharosio, maltosio, lactosio, raffinosio, dextrino, mannitolo, dulcitolo, vel salicino. Gigantea colonia elevata, compacta, superficie fracta, ambitu irregulari; incremento parvo in segmentis radicis *Dauci Carotae* et tuberum *Solani tuberosi*. Vicissitudinem in lacte nec inducit nec gelatinam liquefacit. Segregatus e nectari florum, melle et solo. Pro incremento normali postulat biotinum, acidum pantothenicum, et inositolum.

Zygosaccharomyces rugosus, sp. nov.

In culturis juvenilibus, cellulis ovalibus vel rotundatis, fere 4–6 μ longis, 3–4 μ latis. Regenerat et gemmis et copulatione aequarum cellularum ascosporis deinde orientibus. Ascis 1–4 (plerumque 2–3)-sporis; sporis leviter ovalibus vel rotundatis, fere 3–3.5 μ longis. In diluto forti sacchari crescere potest. Inducit fermentationem alcoholicam in dextrosio, levulosio, mannosio, saccharosio, maltosio sed nullam in arabinosio, xylosio, galactosio, lactosio,

raffinosio, dextrino, mannitolo, dulcitolo, vel salicino. Gigantea colonia rotunda, expansa superficie crasse plicata; incremento amplo in segmentis radicis *Dauci Carotae* et tuberum *Solani tuberosi*. Vicissitudinem in lacte non inducit sed gelatinam tarde liquefacit. Segregatus e melle et solo. Pro incremento optimo postulat biotinum et acidum pantothenicum sed inositolum necessarium non est.

Acknowledgments

The author is indebted to Mr. John Adams and to Dr. Harold Senn, Division of Botany and Plant Pathology, for the Latin descriptions of the two species.

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THE MIGRATION OF FUNGAL NUCLEI IN AN ELECTRIC FIELD[1]

By E. S. Dowding[2] and E. H. Gowan[3]

Abstract

The mycelium of *Neurospora tetrasperma* readily conducts an electric current. During the time an electric current of about 5 μa. is allowed to pass through cultures of *N. tetrasperma* or *Gelasinospora tetrasperma*, mycelial growth ceases almost entirely, but afterwards the fungi grow normally again and show no ill effects. Electric currents of the order of 1 or 10 μa. running in either direction through two fused strains of *N. tetrasperma* do not alter the normal direction of nuclear migration from one strain to the other.

Introduction

In a previous paper (6) it has been shown that in the fungus *Gelasinospora tetrasperma* Dowding, after fusion between two strains, *A* and *B*, of opposite sex, nuclei migrate from strain *A* and pass from cell to cell throughout strain *B*, so that strain *B* comes to contain nuclei of both sexes and develops fruiting bodies. This agrees with the discovery of nuclear migration in the Basidiomycetes by Buller (4, pp. 222–223) and in the Ascomycetes by Dodge (5). In *G. tetrasperma* the behaviour of nuclei is as follows: (1) they travel through the plant at a speed of from 4 to 5 mm. per hr. (about twice the growth rate of the hyphae); (2) they pass from cell to cell via the perforations in the transverse septa; (3) although the direction of nuclear migration between paired strains is fairly constant, it can be changed by light; (4) in a growing hypha the cytoplasm streams vigorously towards the tip. Experiment shows that this stream does not carry the nuclei but leaves them behind attached to the thin layer of cytoplasm lining the cell.

Since cytoplasmic streaming is not responsible for nuclear migration, a search for the mechanism was made elsewhere, in the field of electrical phenomena. Taylor and his co-workers (7), using an exceedingly weak direct current through non-polarizable microelectrodes, were able to observe by dark-field illumination the migration of particles through the cytoplasm of a slime mold. In the heterothallic fungi, if it should happen that the (+) and (−) strains possess a naturally different electrical potential, nuclei may migrate from one strain through the other, due to the same cataphoretic force that carries the protoplasmic particles of the slime mold. Experiments were therefore performed to determine whether or not the direction of nuclear migration could be influenced by an electric field.

[1] *Manuscript received August 23, 1941.*

Contribution from the Provincial Laboratory and the Department of Physics, University of Alberta, Edmonton, Alta.

[2] *Mrs. E. S. Keeping, Provincial Laboratory, University of Alberta.*

[3] *Department of Physics, University of Alberta.*

Material and Methods

To find suitable material for experiment, a search was made for compatible strains of a fungus whose nuclei under ordinary laboratory conditions and good illumination almost invariably migrate in the same direction. Thus strains "25" and "26" of *G. tetrasperma*, which were first tried, were later rejected because it was found that of 60 paired cultures growing under identical lighting conditions 50 produced perithecia on the "25" side alone, and 10 produced perithecia on the "26" side.

Finally *Neurospora tetrasperma* Shear and Dodge strains "X" and "Y" (of opposite sex), kindly sent by Dr. B. O. Dodge, were tested. About 100 pairings were made, and under the proper moisture conditions perithecia always appeared on the *Y* mycelium exclusively. These strains of *N. tetrasperma* were therefore used in the experiments on nuclear migration. For a preliminary experiment on the effect of an electric current on fungal development the homothallic *N. tetrasperma* and *G. tetrasperma* were used.

To produce migration of charged particles through a solution by cataphoresis it is necessary for an electric current to flow through the solution. For example, suppose a gum mastic sol is placed in a Petri dish upon the stage of a microscope. Let copper electrodes that dip into the liquid be connected in series with a galvanometer and with a variable source of direct potential. Then as the current is increased from zero, the particles of gum mastic are seen to travel more and more swiftly across the field of view.

To set up an electric experiment with *N. tetrasperma*, a Petri dish of agar was inoculated at opposite ends with the strains *X* and *Y*. Near each inoculum, a sterile electrode was bent round the rim of the dish and into the agar. The two inocula were allowed to grow out, and after about 24 hr. the aerial mycelium from each had come into contact with an electrode, and also had approached the other mycelium (Fig. 4). Within another 24 hr. the two mycelia had met and fused. Before the two strains met, a current was sent through the dish and allowed to flow until several hours after they had fused. The current was then disconnected and the cultures were stored for another three days, so that they produced fruiting bodies. The living nucleus could not, of course, be observed under the microscope as were the gum mastic particles, but it was expected that any change in the direction of their migration would show itself later in the distribution of the perithecia. The culture medium used throughout the work was Difco corn meal agar.

In the electrical experiments, it was discovered that when the positive electrode was of copper this substance had a deleterious effect on the development of the fungus. After a current had been run for even a few minutes through such a culture, no perithecia whatever developed on the agar in an area that reached from the positive copper electrode for a radius of about 30 mm. (Fig. 1A). However, transfers from this area to fresh agar showed the fungus to be viable. This effect was due probably to copper diffusion. In later experiments the positive electrode was always platinum.

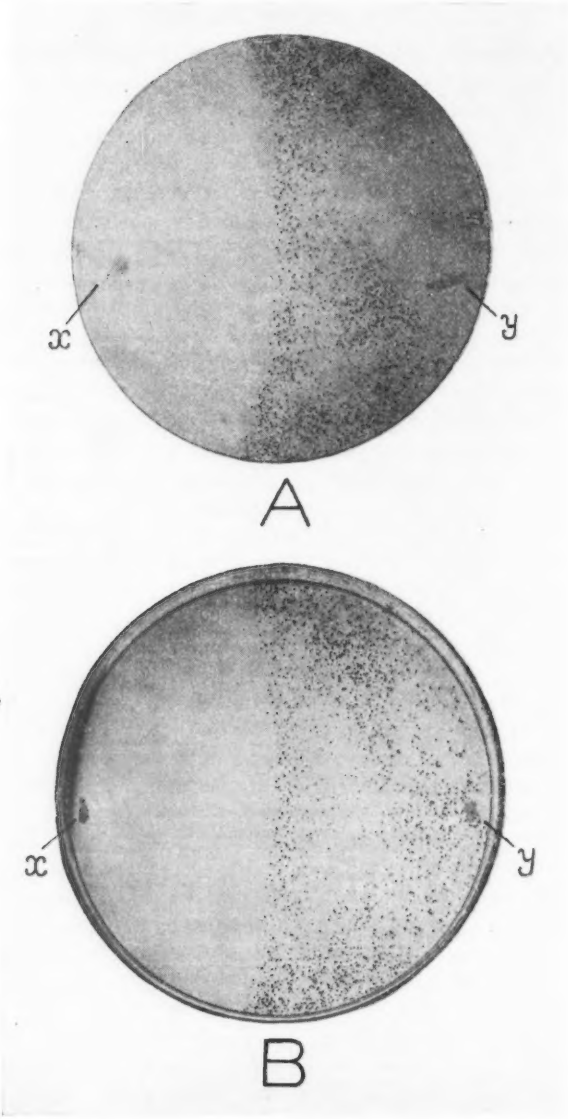

FIG. 1. *Neurospora tetrasperma, strains X and Y, which were subjected to an electric current during the period of nuclear migration. The normal direction of migration (X nuclei into Y mycelium) was unchanged by the current, so that the Y mycelium bears the perithecia;* A, (+) *electrode of copper, which has produced an infertile area near it;* B, (+) *electrode of platinum.*

In the electrical experiments, it was found that the two paired mycelia met and that aerial hyphae came into contact with the electrodes at about the same time and then the galvanometer readings gradually increased. When it happened in an experimental plate that no increase in current was recorded during the second day after inoculation, it was assumed that the growing hyphae had not made proper contact with the electrodes, and this plate was not used for experiment.

Occasionally there were sudden changes in galvanometer reading of from 5 to 20 μa. These jumps were found to be due to faulty contact between electrode and agar, and readings from such cultures were therefore disregarded. In experiments on agar conductivity, the electrodes were firmly secured to the glass dish before the agar was poured in. In Experiment 1, in which the current was run through the agar for a week, the electrodes were made of sheets of lead foil about 2 cm. square and glued to the dish so that the agar as it dried would not shrink from the electrodes.

Five experiments will now be described. The first four are preliminary ones, while the last deals with nuclear migration.

Conductivity Experiments

Experiment 1: Sterile Agar

A Petri dish which had been fitted with electrodes and which was filled with agar, was left on a table where the agar gradually dried at room temperature. The two electrodes were connected with a source of direct current, which gave a constant potential of 1.6 v., and with a galvanometer. The resistance was approximately 30,000 ohms. Galvanometer readings were made one to four times daily for about a week, and are recorded in the following table.

TABLE I

CONDUCTIVITY OF STERILE AGAR DURING DRYING

Elapsed hours	Current, μa.	Elapsed hours	Current, μa.
25	18.5	96	17.7
26	17.4	100	17.8
28	16.6	106	17.9
33.5	17.3	120	17.7
47.5	17.4	124	17.7
53	17.6	144.5	17.7
56	17.8	148	17.8
72	17.7	168	17.7
76	17.7		

It may be seen that the conductivity of the agar remained remarkably constant while the experiment was running, the current varying within 1 μa. from the mean.

This experiment was repeated with two more agar plates and similar results were obtained.

Experiment 2: Agar with Mycelium

A Petri dish fitted with electrodes was filled with agar and inoculated in the centre with a mixture of strains *X* and *Y* of *N. tetrasperma*. The electrodes were connected with a source of direct current and a galvanometer. Galvanometer readings were made three or four times daily for three days, that is, during the time the mycelium was growing out over the agar but before it made contact with the electrodes. The average of the 12 readings was 14.7 μa. and none of the readings varied from this more than 0.8 μa.

On the fourth day the mycelium had reached the sides of the dish and grown into the air where it came into contact with the electrodes. The galvanometer reading then rose to 17.5 μa.

Experiment 3: Mycelium Alone

In each of six Petri dishes of agar, two agar strips were cut out so that there would be a double gap in the path of an electric current passing through the plate (Fig. 2). The glass surfaces of the dishes exposed when the agar

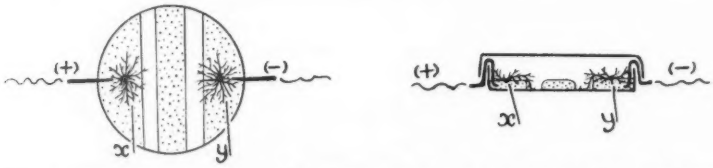

FIG. 2. *Surface and side views of N. tetrasperma cultures used in conductivity experiment.*

was removed were thoroughly dried with sterile cotton wool to reduce possible current leakage to a minimum. Each plate was fitted with electrodes and inoculated with *N. tetrasperma* strains *X* and *Y*. The mycelia soon grew out and extended over the glass barriers. A constant potential of 420 v. was applied and galvanometer readings were taken (1) while the paired mycelia were about 1 cm. apart, and (2) after fusion. Table II shows that, in almost all the dishes no current passed until the two mycelia fused, after which the readings ranged from 10 to 52 μa.

TABLE II

CONDUCTIVITY OF MYCELIUM

Plate number	Current, μa.	
	Before fusion	After fusion
1	0	52
2	4.2	22
3	0	38
4	0	17
5	0	10
6	0	27

The Effect of an Electric Current on Fungal Development

Experiment 4

The normal growth rate of *N. tetrasperma* was first determined as follows. An inoculum was allowed to grow outwards from the centre of the agar in a Petri dish, and then the periphery of the mycelium was traced with ink on the under surface of the dish. During the next eight hours a similar ring was drawn every two hours. By treating several cultures by this method, the growth rate of the mycelium was found to be about 1.5 mm. per hr.

For the electrical experiment, two Petri dish cultures of *N. tetrasperma* (homothallic strain), ringed as before, were placed in a glass-topped box containing a saturated atmosphere. Platinum wires dipped into the agar served as negative electrodes. The positive electrodes were sharpened copper wires fixed about 2 cm. above the mycelium (Fig. 3). The lid of one culture was removed, allowing the current to pass through the mycelium. The lid of the second (control) culture was left on, screening the mycelium from the current. A potential of 5000 volts was applied to the electrodes.

The current through the mycelium was calculated as follows. The leakage current around the box was 0.6 μa.; the current through the electrode above the covered dish was 2.1 μa.; that through the electrode above the open dish was 4.7 μa. Thus about 2 μa. went through the tips of the aerial hyphae to the agar and thence to the negative electrode.

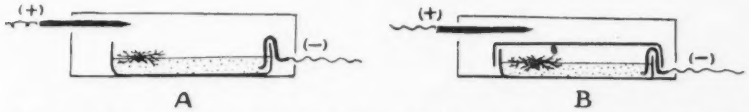

FIG. 3. *Cultures of N. tetrasperma used in electric current experiment;* A, *exposed culture;* B, *control.*

In order to calculate approximately the total cross sectional area of fungus through which the current passed, the procedure was as follows. An inoculum of *N. tetrasperma* placed at one end of a Petri dish of agar, was allowed to grow out over a film of water spread over a microscopic slide which just fitted inside the dish and lay on the agar. After a sheet of mycelium had covered the slide it was cut away from the agar and gathered into a roll of a length equal to the width of the slide. The diameter of the cylinder of mycelium was 1 mm., which is only 5% greater than the effective diameter, i.e., the diameter of a solid cylinder having the same cross sectional area as the actual cylindrical bundle of hyphae, the individual threads in the bundle being supposed for the purposes of calculation to be circular in section and packed as tightly as possible. A current of 2 μa. had passed through the cylinder; this represents about 2.6 μa. per mm.^{2} of mycelium.

After the current had run for four hours it was disconnected and the cultures were ringed again. It was found that the growth of the mycelium in the experimental plate during the four hours was practically negligible,

while the control had grown 7 mm. (about the normal rate). The growth of the experimental culture was measured again for several hours after the current had been disconnected. It was found that normal growth rate was resumed immediately after the current had ceased to flow, and in a few days perithecia formed normally.

A similar experiment was carried out using *G. tetrasperma* and similar results were obtained.

The conclusion is that a current of about $2\frac{1}{2}$ μa. per mm.^{2} through the mycelium retards growth while it is flowing, but has no permanently injurious effect either on growth or reproduction.

In Experiment 5 on nuclear migration the current was run after growth had ceased. Although the current that passed through the mycelium was probably 10 times greater than that which in Experiment 4 had been found to arrest growth, the fungus suffered no ill effects and produced perithecia normally.

Blackman and Legg (1) working with pot cultures of cereal seedlings, found that an electric current of the order of 0.01 μa. per plant retarded growth. The barley coleoptiles which they used measured about 0.013 cm.^{2} in cross section (2), so that the current per mm.^{2} must have been 0.008 μa., or about 1/300 of the current in this experiment.

Discussion

In the past, numerous experiments have been carried out to determine whether or not the growth and yield of a field of crop plants could be increased by the presence of an electrically charged network over it. Such "Electroculture" experiments carried out in England showed a marked increase in yield, but in the United States similar experiments did not confirm this finding (3). It would be a comparatively simple undertaking to extend the electrocultural investigations by experiments (similar to the one described above) on fungi such as *Neurospora* and *Gelasinospora*, which are small and easy to culture, and complete their life history in less than a week.

The Effect of an Electric Field on Nuclear Migration

Experiment 5

Fourteen Petri dishes were inoculated at opposite ends with *N. tetrasperma* strains *X* and *Y*. Eight of the plates served as controls, while the remaining six were fitted with electrodes and used for experiment as in Fig. 4. In

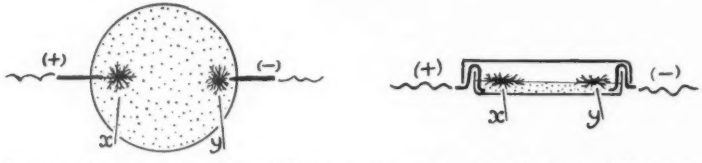

FIG. 4. *Surface and side views of N. tetrasperma cultures used in electric field experiment.*

one-half of the experimental plates the *X* inoculum was near the (+) electrode and in the other half it was near the (−) electrode. To eliminate light as a cause of any possible change in the direction of nuclear migration, the *Y* mycelium, into which *X* nuclei normally migrate, was always placed nearest the light.

The day following inoculation the electrodes were connected with a 420 v. power supply and a galvanometer, the cultures being connected in series. At this time the current through each Petri dish was 11 μa. Current readings taken at intervals, from just before fusion until shortly after fusion was complete in all the plates, gradually increased from 13.7 to 22 μa., as shown in Table III.

TABLE III

CONDUCTIVITY OF SIX CULTURES DURING PERIOD OF FUSION

Elapsed hours	Current, μa.	Remarks
18	13.7	Mycelia not in contact
22	14.5	One pair of mycelia in contact
24	18	All pairs in contact
27	19.3	Fusion probably commencing
29	22	Fusion probably complete
31	22	No change

After the current had been running for two days it was disconnected, and the cultures were stored for another five days, by which time they had produced perithecia.

In the eight control and the six experimental plates the perithecia had developed on the *Y* mycelium exclusively (Fig. 1B). Nuclei must have migrated always from the *X* to the *Y* mycelium, irrespective of the direction of the electric current.

A second experiment was carried out, this time using a lower potential so that the current per plate was of the order of 1 μa. Six cultures were used. In three plates the current travelled in one direction, and in the other three in the opposite direction. Galvanometer readings during fusion rose from 0.6 to 0.9 μa. When the cultures produced perithecia their distribution (on the *Y* mycelium) showed that the current had caused no change in the direction of nuclear migration in any of them.

Discussion

The experiments of running an electric current in either direction through fusing strains of *N. tetrasperma* produce no change in the normal direction of nuclear migration. Yet it cannot be assumed from this that cataphoresis is not the mechanism of nuclear migration. It is impossible to say whether or not under the conditions of the experiments the fungal cytoplasm was actually in an electric field. It is possible that the electric current, instead

of travelling through the cytoplasm, passed along the cell walls of the hyphae or even along the film of water condensed upon them. Even supposing that the current passed through the cytoplasm, the amount of current used (1 and 10 μa.) may not have been the effective strength for cataphoresis of nuclei.

Again, since it is already known that light readily influences the direction of nuclear migration in *Gelasinospora* (6), it may be that *Gelasinospora* would have been more suitable than *Neurospora* for electrical experiments. However, since *Gelasinospora* grows best in very humid conditions, there would be the difficulty of leakage currents through water films to be overcome. Further, since the direction of nuclear migration in *Gelasinospora* is normally inconstant, many more experiments and controls would be needed than in *Neurospora* to establish the significant effect of an electric field.

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BIOLOGICAL DECOMPOSITION OF CHEMICAL LIGNIN

III. APPLICATION OF A NEW ULTRA-VIOLET SPECTROGRAPHIC METHOD TO THE ESTIMATION OF SODIUM LIGNOSULPHONATE IN CULTURE MEDIA[1]

By G. A. Adams[2] and G. A. Ledingham[3]

Abstract

An ultra-violet spectrographic method has been developed for estimating the amount of sodium lignosulphonate present in solutions. The method has been applied to the measurement of lignosulphonate losses in liquid media after fungous growth and the results are compared with those obtained under similar conditions by the β-naphthylamine precipitation method. The spectrographic method gave slightly lower values but was free from certain errors present in the chemical method. The results confirmed previous observations that fungi are capable of decomposing lignosulphonates.

The quantitative estimation of lignosulphonates presents several difficulties. Since they do not crystallize, they cannot be separated and directly weighed, neither are there characteristic groups occurring in them in constant amounts which can be used as a basis for estimation. Attempts to use the methoxyl group have not been entirely satisfactory. The methods of estimation hitherto used most frequently have been by precipitation, using 80% ethanol or methanol, calcium oxide, lead acetate, sodium chloride, calcium chloride, sulphuric acid, or β-naphthylamine. The most efficient of these has been found to be β-naphthylamine (7).

In the course of a study on the decomposition of lignosulphonate solutions by fungi, it was necessary to measure accurately the lignosulphonate in the culture media before and after fermentation. These measurements were made by a method employing β-naphthylamine precipitation (1). However, incomplete precipitation leaves room for inaccuracies, especially in media where fungi have been growing and consequently have brought about changes in the medium as compared with the uninoculated controls. In experiments with wood destroying fungi there were instances where the β-naphthylamine precipitate actually weighed more than that of the controls (6).

In searching for a more reliable and accurate method for determining sodium lignosulphonate, a method was developed using quantitative measurements of the absorption spectrum of sodium lignosulphonate in the ultra-violet region. The ultra-violet spectrum of lignin and its derivatives has been studied extensively (2, 3, 4, 8) and it has been established that the lignin derivatives of softwoods have a band in the ultra-violet with the maximum between 2800 Å and 2870 Å. Cellulose and other carbohydrates do not exhibit any light absorption in this ultra-violet region (5). Conse-

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quently it is possible to measure the ultra-violet absorption spectrum of lignin even in the presence of carbohydrates. The absorption in the region of 2800 Å appears to be characteristic of a main molecular structure and not of some specific groups, and a quantitative measurement of the light absorption can be interpreted in terms of the amount of lignosulphonate in the solution.

Apparatus

The absorption spectra were determined photographically using a Hilger medium quartz spectrograph with a Hilger Spekker ultra-violet photometer. The slit width was set at 0.03 mm. The light source was a spark between tungsten steel electrodes supplied by an oscillating current from 0.25 mf. and 2 mh. fed by a d–c. rectifier giving 18 ma. at 12,000 v.

Eastman No. 40 spectrum analysis plates, developed in Eastman *D*72, were used throughout, as their high contrast made the picking out of points of equal intensity a relatively easy task.

Cells 2 cm. long were used for holding the solution and solvent. These were fitted with quartz windows at each end, held in place by a screw cap.

Technique

Exposures were taken at varying apertures as read by the density drum calibrated in 0 to 1.5 density units. The time of exposure was varied with the drum reading. About a three-second exposure was sufficient for a density reading of zero.

One cell was filled with control solution and the other with control solution to which sodium lignosulphonate had been added. The spectrograms of the two solutions were taken simultaneously so that minor variations in the light source had no effect on the results.

Development of Method

Spectrum Characteristics

It was first necessary to determine whether the sodium lignosulphonate had a spectrum characteristic of lignin derivatives, and whether the nutrients used along with the sodium lignosulphonate in the culture media had spectra that would mask or interfere with the lignosulphonate spectrum. Spectra were taken of a series of varying concentrations of sodium lignosulphonate in water. It was found that a concentration of 0.005% was most suitable for this study. The spectrograms showed sodium lignosulphonate to have a well defined absorption band with a maximum absorption at 2800 Å corresponding to the spectrum of lignin derivatives in general.

Preparation of the Solutions

A number of the common nutrients used for fungal growth were next tested to find if their ultra-violet spectra showed absorption in the same region as the lignin compounds. The solutions tested were 1% ammonium nitrate, 1% potassium acid phosphate, 0.5% magnesium sulphate, 1%

potassium chloride, 1% dibasic ammonium phosphate, 1% ammonium carbonate, trace of ferrous sulphate, and 3% glucose. Sodium lignosulphonate was run on the same plate to facilitate easy comparison. The plates were taken with three seconds' exposure, density zero. It was found that ferrous sulphate was slightly absorbing and ammonium nitrate heavily absorbent, so fungi that grew well without iron in the medium and that did not require ammonium nitrate as a source of nitrogen had to be selected. It was now possible to select from the remainder, nutrients that would make a satisfactory control. A suitable culture medium was made up containing:—

Dextrose	20.0 gm.
Dibasic ammonium phosphate	5.0 gm.
Magnesium sulphate	1.0 gm.
Potassium chloride	1.0 gm.
Sodium lignosulphonate	37.5 gm.
Water	1000 cc.

Another spectrographic control solution was made up which contained all the above constituents except lignosulphonate. The two solutions were sterilized with steam for 15 min. at 15 lb. pressure. The control solution turned a light brown colour during sterilization. To demonstrate that the colour change does not affect the spectrum in the band studied, the following check was made. Both solutions were diluted equally, the sodium lignosulphonate concentration in the one being 0.005%. Spectrograms of the following solutions were then taken with zero density and three-second exposure: (1) the sterilized nutrients against water; (2) the sterilized nutrients against sterilized media. Using water as a check, it was demonstrated that the sterilized nutrients have no absorption band in the region at 2800 Å, while the sterilized medium has a pronounced absorption. This shows that the absorption in the medium is due entirely to the sodium lignosulphonate and not to the other nutrients.

Preparation of Standards

In order to convert the spectrographic data obtained from the spectra of the lignosulphonate culture medium into actual amounts of lignosulphonate existing in the medium, it was necessary to have a set of standards prepared in which the density reading (D) at the wave length of maximum absorption was known for a series of various concentrations of lignosulphonate. A standard culture medium containing 37.5 gm. of sodium lignosulphonate diluted with water to give a 0.005% solution was used. This was photographed in the ultra-violet, along with a similar solution with no lignosulphonate as control. This original solution was diluted to produce sodium lignosulphonate contents of 95, 90, 85, 80%, respectively, on which densities were determined as shown in Fig. 1.

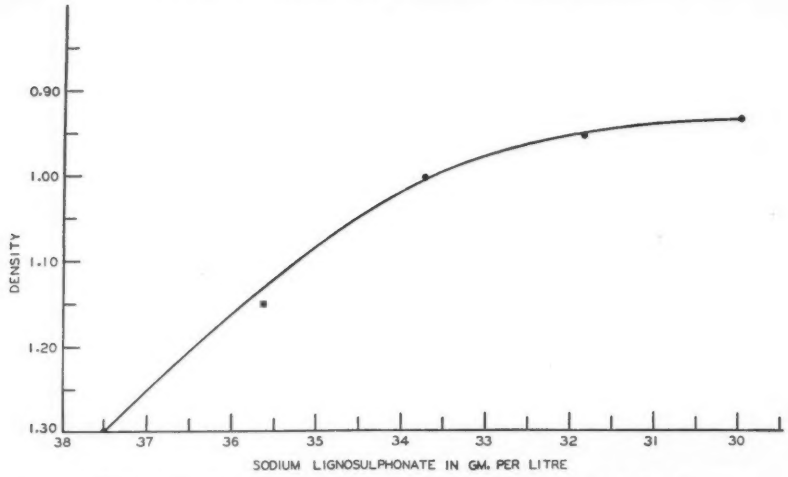

FIG. 1. *Graph relating concentration of sodium lignosulphonate to density.*

Method

The culture medium was made up, sterilized, and pipetted in 100 ml. lots into six flasks of 500 ml. capacity. Three of the flasks were inoculated with spore suspensions of the fungus and three were not inoculated but kept as controls to find whether or not the medium underwent changes without microbial growth. The cultures and controls were incubated in the dark at 25° C. for three weeks.

At the end of the growth period the fungus mat was filtered from the solution and washed thoroughly with water until all traces of the medium had been removed. The washings were added to the original filtrate and the whole made up to a volume of 200 ml. From this solution 0.66 ml. were diluted to 250 ml. for spectrographic analysis. A control was made up from the sterilized nutrient salts without the sodium lignosulphonate.

The spectrograms were taken using 21 density readings from 0.70 to 1.40 with exposure times varied from 10 sec. for density 0.70 to 44 sec. for density 1.40. These densities gave a sufficiently well defined absorption curve to facilitate selection of the point of maximum absorption. The density reading at the peak of the absorption was then transposed to the graph (Fig. 1) relating density and concentration of sodium lignosulphonate and the amount of the lignosulphonate in the solution was read off directly. Since the amount in the original culture medium was known, the loss due to decomposition was readily calculated.

Application of the Method

The culture medium on which *Fusarium culmorum* (W. G. Smith) Sacc. had been grown was analysed at the end of 20 days by the β-naphthylamine

precipitation method and by the spectrographic method described above. The loss in lignin shown by the two methods was as follows:—

Precipitation method	7.82%
Spectrographic method	6.94%

A spectrographic examination of the uninoculated culture medium after a period of three weeks indicated that it had remained unchanged in so far as the spectrum of the sodium lignosulphonate was concerned.

Another experiment was carried out in which *Alternaria* sp. (*T–S–29*) was used for the breakdown of the sodium lignosulphonate. The organism was grown for a period of three weeks and produced a thick heavy mat. The loss in lignin from this culture as found by the two methods of analysis is as follows:—

Precipitation method	6.42%
Spectrographic method	5.02%

Analytical Results on Wood Destroying Cultures

It has been previously pointed out that culture media on which wood destroying fungi have been grown, when treated with β-naphthylamine reagent to precipitate the lignosulphonate, form a precipitate which is not a hard, dark plastic as in the control, but is a coarse dark red powder (6). This type of precipitate weighs more than its corresponding control from an uninoculated culture and gives the apparent result of the lignin concentration being increased during the growth of the fungus. It seemed likely that a spectrographic investigation of this problem would throw more light on the fate of the lignosulphonate during the growth period.

The culture medium was prepared in the same way as outlined previously. The wood destroying organisms, *Poria subacida* (Peck) Sacc. and *Polyporus dichrous* Fr., were grown in duplicate with two uninoculated control flasks. The analyses were carried out at 47 days when *Poria subacida* showed small scattered colonies in all duplicate cultures while *Polyporus dichrous* had heavy submerged spongy mats. The lignin content was determined both by the β-naphthylamine precipitation method and the spectrographic method, and the results are shown in Table I.

TABLE I

RESULTS OF SODIUM LIGNOSULPHONATE ANALYSES ON MEDIA AFTER GROWTH OF WOOD DESTROYING FUNGI

Sample	Lignosulphonate decomposed, %	
	Chemical method	Spectrographic method
Poria subacida (1)	(Gained in wt.) 23.12	2.93
Poria subacida (2)	(Gained in wt.) 17.71	4.53
Polyporus dichrous (1)	2.19	4.53
Polyporus dichrous (2)	0.31	4.53

It can be seen from the results in Table I that when the lignosulphonate is determined by the chemical method for *Poria subacida* cultures, there is a marked increase in weight of the precipitate over the uninoculated control, thus giving an apparent increase in the lignosulphonate of the medium. On the other hand the spectrographic results, which are free from the errors of the chemical method, indicate small but positive decomposition of the lignosulphonate. It is apparent that some product of metabolism formed in the growth of the fungus is precipitated with the lignosulphonate or else some change has taken place in the medium that renders more of the lignin precipitable by β-naphthylamine, 68% normally being precipitable.

Although *Polyporus dichrous* cultures showed a positive loss in lignosulphonate when analysed by both methods, the spectrographic results were considerably higher. The fact that the nature of β-naphthylamine precipitate from these cultures was beginning to change to the same type as found in the cultures of *Poria subacida* indicates that the same phenomenon is taking place but has not progressed so far. If the growth period had been prolonged, an apparent increase in lignosulphonate would have occurred. This indicates

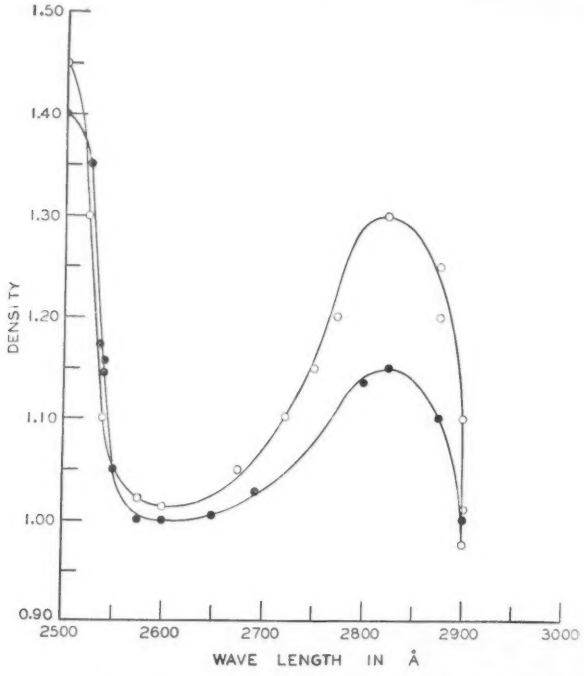

FIG. 2. *Absorption spectra curves of sodium lignosulphonate.* —○—○— *Uninoculated solution.* —●—●— *Solution on which Fusarium culmorum had been grown for 20 days.*

that the precipitation method does not give a true measurement of lignosulphonate decomposition, especially in cultures that have been growing for a long period of time.

Discussion

The absorption spectra of almost all lignins that have been studied show an absorption band at about 2800 Å. It has been possible to make use of this specific absorption in solutions of sodium lignosulphonate to measure the amount of the substance present before and after breakdown by microorganisms. The form of the absorption curve from the inoculated cultures is in strict conformation to that of the uninoculated control solution, although the amount of absorption varies as shown in Fig. 2. Apparently the lignosulphonate that has been broken down by the organism has been changed into a substance or substances that have no interfering absorption in this region of the ultra-violet. It appears likely, therefore, that the molecule of sodium lignosulphonate has been wholly converted to some simpler molecule, rather than partially modified into another related substance. If it were changed slightly in its chemical composition, the absorption curve would not be expected to remain identical in form with that of the uninoculated lignosulphonate medium. Examination of uninoculated lignosulphonate culture medium shows that it does not undergo any changes in so far as its spectral properties are concerned, even in 50 days, provided it is stored in the dark.

Acknowledgments

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RUST REACTIONS OF CHINESE WHEAT VARIETIES AND CERTAIN CANADIAN HYBRID STRAINS[1]

By Yun-Chang Wang[2]

Abstract

About 160 Chinese spring wheats and a number of Chinese winter wheats were tested in the seedling stage in the greenhouse to determine their reaction to eight physiologic races of stem rust (*Puccinia graminis Tritici*) and to four physiologic races of leaf rust (*Puccinia triticina*). The spring wheats were also subjected, in the field, to epidemics of stem rust and leaf rust in which a large number of physiologic races of each rust were employed. The wheats were classified as one or another of the following species: *Triticum vulgare*, *T. compactum*, *T. durum*, and *T. turgidum*.

By means of these tests, it has been demonstrated that the Chinese wheats are rather highly susceptible to the physiologic races of stem rust prevalent in North America. Many of the wheats showed, however, considerable resistance to leaf rust in both the greenhouse and the field tests. Varieties resistant to leaf rust were present in all of the four above-mentioned species.

Seventy-five lines of Canadian spring wheat derived from a cross between Renown Selection, which is resistant to stem rust and moderately resistant to leaf rust, and Garnet, which in North America is resistant to stripe rust (*Puccinia glumarum*), were tested in the seedling stage in the greenhouse for their reaction to stripe rust. Two physiologic races (race 6 and race 13) were used in these tests. The same lines were subjected to rust epidemics in the field to determine their resistance to stem rust and leaf rust. Several of these lines were found to possess resistance to all three rusts. These lines may prove valuable breeding material in case it is desired to develop by breeding methods Chinese varieties resistant to all three of these rusts.

Introduction

No reliable record exists of the losses caused by cereal rusts in China, but there can be no doubt that these are often severe. In the northern part of Honan, in north China, the writer has noticed that, in years of abundant moisture and heavy stand of grain, the damage caused by rust was particularly severe. In certain years, when normal yield was expected, he has seen an outbreak of rust reduce the yield to 20 or 30% of that anticipated. Farmers in that area consider this disease as the worst of all the troubles of the wheat crop, with the possible exception of drought, which occurs frequently in north China.

Wheat, in China, is parasitized by three separate species of rust, namely, stem rust (*Puccinia graminis Tritici* Erikss. & Henn.), leaf rust (*Puccinia triticina* Erikss.), and stripe rust (*Puccinia glumarum* Erikss. & Henn.). As far as the writer has been able to discover, the two last-mentioned rusts cause the most serious damage.

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Contribution No. 681, from the Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada.

[2] *The work was carried out at the Dominion Laboratory of Plant Pathology, Winnipeg, Man., during the author's tenure (1938–1939) of a fellowship granted him by the China Foundation for the Promotion of Education and Culture.*

The present work was undertaken as a result of the writer's realization of the severity of the losses caused by these rusts and the necessity of breeding varieties resistant to them. Such a project can only be accomplished by utilizing and applying the principles of plant breeding and plant pathology, as has been done with conspicuous success in North America and elsewhere, through the close co-operation of plant breeders and plant pathologists. In the present work, the writer has attempted to discover the relative resistance of different wheat varieties that might be of value for a breeding program. In an attempt to attain this object two different methods have been pursued. The first was to try to find the necessary rust resistance among Chinese wheat varieties. This has been attempted by testing a large number of Chinese wheats for their resistance in the greenhouse and in the field to many physiologic races of leaf rust and stem rust. In this work, it was unfortunately not possible to test the resistance of the wheat varieties to stripe rust as this rust does not occur in the central part of North America, where the work was conducted, and was not available for greenhouse studies during most of the period when the work was in progress. The second method employed was to try to discover, among foreign wheats, varieties that possess resistance to the three above-mentioned rusts. Such varieties would be useful as breeding material even if they were not themselves of value under conditions in China. In this category may be included certain lines recently developed at the Dominion Rust Research Laboratory from a cross between Garnet, which in North America is resistant to stripe rust (6), and Renown, which is resistant to stem rust and fairly resistant to leaf rust (5, 7). If some of these lines were found to be resistant to all three rusts, they would be extremely useful for purposes of crossing with Chinese wheat varieties. For this reason greenhouse tests were conducted with 75 of these lines to determine their resistance to stripe rust, and field experiments were carried out to test their resistance to stem rust and leaf rust.

The problem of determining the resistance of any wheat variety to stem rust, leaf rust, or stripe rust is complicated by the fact that each of these rusts is not a unit, but consists of many different strains known as physiologic races. To determine the resistance of any variety to one of these rusts, it is necessary to test its resistance not only to one but to many physiologic races. In the present work, it would have been desirable to test the resistance of the Chinese wheats to physiologic races collected in China. This, however, was not possible. In the absence of rust from China, the varieties were tested for their resistance to several physiologic races that occur commonly in North America. Two of the races of stem rust used in these tests (races 9 and 15) have been reported by Tu (10) as occurring in China. Probably these tests furnish an indication of the resistance of wheats to the stem rust races that occur in China, although only six races so far seem to have been identified in that country.

Materials and Methods

About 200 Chinese wheats were tested in the greenhouse and in the field to determine their resistance to stem rust and leaf rust. These wheats were obtained by the writer from the United States Department of Agriculture through the kindness of Dr. S. C. Salmon, agronomist in charge of wheat investigations. They were originally obtained, for the most part, from the University of Nanking, Nanking, China, and represent nearly all the wheat growing regions of China. These wheats, with a few exceptions, were of spring habit.

Owing to the fact that identification of wheat varieties can be made with certainty only in the adult stage of growth, it was possible to identify only the varieties that were grown to maturity in the field. Among the varieties that could not be identified are all the winter wheats, and certain spring wheats of which the quantity of seed received was only sufficient for seedling tests. The great majority of the Chinese wheats studied were identified as one or another of the four species, *Triticum vulgare* Host, *T. turgidum* L., *T. durum* Desf., *and T. compactum* Host.

The bulk of the Chinese wheats, 165 samples, were identified as common wheat, *T. vulgare*. These had their origin in nearly every province in China.

The rivet wheats, *T. turgidum*, in this collection, were limited to two samples, one from Kansu and one from Sinkiang (Chinese Turkestan).

Eight of the 14 specimens of *durum* wheat, *T. durum*, originated from Yunnan, the remainder, from Kansu, Szechwan, Honan, and Sinkiang.

There were also included in this collection 14 club wheats, *T. compactum*, eight from Szechwan, three from Kansu, and three from Yunnan.

Two different kinds of tests were employed to determine the resistance of the Chinese wheats to stem rust and leaf rust: (1) greenhouse tests in which known physiologic races were employed, and (2) field tests in which the wheats were subjected to natural rust infection plus artificially induced epidemics of both rusts. In the greenhouse tests, varietal resistance was judged by the reaction to rust of seedling leaves inoculated with pure cultures of physiologic races. For the tests with stem rust, eight physiologic races were employed, namely, races 9, 15, 19, 21, 34, 38, 51, and 56. In the tests with leaf rust, races 1, 9, 58, and 76 were used. The field experiment comprised separate tests for each of the two rusts, the varieties, in both tests, being planted in five-foot rows surrounded by border rows of the susceptible wheat Little Club. On these border rows an epidemic was induced artificially by dusting spores of many physiologic races over the plants by the use of a duster made specially for this purpose.

For the test with stripe rust, seed of 75 lines of Canadian wheat was obtained from Dr. R. F. Peterson, of the Dominion Rust Research Laboratory, Winnipeg, Man. These were F_4 lines from a cross between Renown Selection (R.L. 716.6) and Garnet. Of these parents the former is resistant to both stem rust and leaf rust while the latter has considerable resistance to stripe

rust and matures very early. The tests of the stripe rust reaction of these hybrid lines were conducted in the greenhouse by inoculation of seedlings with physiologic races 6 and 13.

The commonly accepted system of recording infection types of cereal rust on seedling plants was adopted in recording the different rust reactions in the greenhouse tests. For stem rust of wheat, the system of Stakman and Levine (9) was used; for leaf rust of wheat, that of Mains and Jackson (4); and for stripe rust that of Hungerford and Owens (2).

For recording the severity of the infection on the adult plants, the scale given by Clark, Martin, and Stakman (1) of the Office of Cereal Crops Diseases, United States Department of Agriculture, was used. According to this scale, rust severity is recorded as varying from a trace to 100%. Those plants with very few pustules are recorded as having a trace of rust and those with maximum infections as having 100% severity.

Reaction of Chinese Wheats to Stem Rust and Leaf Rust

The results of the tests conducted in the greenhouse and in the field to determine the reaction of Chinese wheats to stem rust and leaf rust are summarized in Table I.

The tests of the reaction of 184 Chinese wheats to the eight physiologic races of stem rust (races 9, 15, 19, 21, 34, 38, 51, and 56) showed that not one of these wheats possessed resistance in the seedling stage to all eight races, although a number of wheats were resistant to one or more of the races. Among wheats of the *vulgare* type, no variety was found to be resistant to more than three of the eight races. In *T. durum* and in the winter wheats, the proportion of varieties possessing some resistance was greater than in the *vulgare* wheats of spring habit. The lack of stem rust resistance in the Chinese wheats was confirmed by the field test in which all of the 189 wheats tested proved susceptible.

Considerably more resistance was shown by the Chinese wheats to physiologic races of leaf rust. In *T. vulgare* several varieties of diverse morphologic types showed resistance in the seedling stage to the four races employed in the greenhouse test (races 1, 9, 58, and 76). Most of the wheats resistant in the greenhouse tests were also resistant in the field test, but several wheats that had proved susceptible in the seedling stage showed resistance in the field. Consequently the proportion of resistant wheats was markedly larger in the field test. This disparity between the two tests would indicate the presence among some of the Chinese varieties of mature plant resistance to leaf rust.

Reactions of Certain Canadian Spring Wheats in the Seedling Stage to Two Physiologic Races of *Puccinia glumarum*

It would have been desirable to determine the reactions of the Chinese wheats to stripe rust (*P. glumarum*) as well as to stem rust and leaf rust. As already stated, this was not possible as stripe rust was not available

TABLE I

REACTION, IN THE GREENHOUSE AND IN THE FIELD, OF CHINESE WHEATS TO STEM RUST AND LEAF RUST

Species of wheat tested	Greenhouse reaction							
	Stem rust				Leaf rust			
	Number wheats tested	Number S or MS to all eight races	Number R or MR to one or more races	Number R or MR to all eight races	Number wheats tested	Number S or MS to all four races	Number R or MR to one or more races	Number R or MR to all four races
Spring wheats								
T. vulgare	142	124	18	0	138	98	31	9
T. compactum	10	8	2	0	10	3	7	0
T. durum	8	5	3	0	7	6	0	1
T. turgidum	1	1	0	0	1	0	1	0
T. spp.	2	1	1	0	1	1	0	0
Winter wheats	21	12	9	0	23	14	8	1
Total	184	151	33	0	180	122	47	11

Species of wheat tested	Field reaction (Natural and artificially induced epidemic)							
	Stem rust				Leaf rust			
	Number wheats tested	S	MR	R	Number wheats tested	S or MS	MR	R
Spring wheats								
T. vulgare	157	157	0	0	162	81	39	42
T. compactum	16	16	0	0	13	2	1	10
T. durum	14	14	0	0	12	0	8	4
T. turgidum	2	2	0	0	2	0	0	2
T. spp.	–	–	–	–	–	–	–	–
Winter wheats	–	–	–	–	–	–	–	–
Total	189	189	0	0	189	83	48	58

Explanation of symbols:—
S = susceptible; MS = moderately susceptible; MR = moderately resistant; R = resistant.

during most of the period of the greenhouse tests. In the spring of 1939, two physiologic races of stripe rust (races 6 and 13) were brought into culture in the greenhouse. At this time, it was too late to commence tests with the Chinese wheats.

As certain Canadian wheats, which were known to be resistant to stem rust and leaf rust, were also thought to possess resistance to stripe rust, the writer decided to test these for their resistance to the two races of stripe rust, because any of these strains found resistant to all three rusts would be of great value in any Chinese plant breeding program designed to develop rust resistant wheats. Seventy-five such lines were included in the test. These

lines were derived from F_3 plants, which appeared to be resistant to stem rust and leaf rust in field tests in 1938. All of the lines were tested for their reaction to race 6, but, owing to shortage of inoculum, it was possible to test only 25 of the lines for their reactions to race 13. Of these 25 lines, five were resistant to both races, seven were resistant to race 13 and moderately resistant to race 6, while one line was moderately resistant to both races. Of the remaining 50 lines which were tested to race 6 alone, 20 showed immunity or resistance. As these lines are resistant to stem rust and as most of them proved resistant to leaf rust in a field test conducted in the summer of 1939, they would appear to hold considerable promise as breeding material.

Discussion

Although wheat has been cultivated in China for a very long time, it is only in recent years that scientific attention has been given to the botanical or agronomic characteristics of Chinese wheats or to the diseases to which they are subject. Of these diseases the rusts are perhaps the most important. The desirability of developing wheat varieties resistant to rusts can therefore not be questioned. As all three of the rusts of wheat, stem rust, leaf rust, and stripe rust, occur in China, the problem of developing rust resistant wheats becomes one of combining in a single variety resistance to all of the three rusts.

It seems clear that the first step towards the attainment of this objective is to discover to what extent existing Chinese varieties are resistant to these rusts. The present work has been largely devoted to this task. In this work, about 200 Chinese wheats have been tested for their resistance to some of the most prevalent physiologic races of stem rust and leaf rust in North America. For reasons stated elsewhere in this paper it was not possible to test the resistance of these varieties to stripe rust.

No country that has attempted to solve its rust problems by plant breeding methods depends, however, entirely on its own varieties. On the contrary, wherever large-scale plant breeding programs have been undertaken, varieties with the desired qualities have been gathered together with the purpose of combining these qualities with those of varieties of local origin in an attempt to create new varieties suitable to the particular climatic and soil conditions of any given country. Any plant breeding program organized in China must therefore be based on the best available genetic material, whether this be of local or of foreign origin. It was with this idea in mind that the present work was extended to include tests of the resistance to stripe rust of certain Canadian spring wheat lines known to possess resistance to stem rust and leaf rust and thought to possess also resistance to stripe rust.

The development of these lines is worth brief consideration as it illustrates the methods used by Canadian plant breeders in combining in one variety resistance to several rusts. The lines originated in a cross between Renown and Garnet. In Renown is combined (in the adult stage of growth) high resistance to stem rust and a considerable resistance to leaf rust (5, 7).

Garnet, while susceptible to stem rust and leaf rust (5, 7), is resistant to stripe rust (3, 6, 8). In the F_2 and F_3 generations of the cross, the lines resistant to stem rust and leaf rust were selected but, as stripe rust does not occur in Manitoba, where the work was done, no selection could be made for resistance to that rust. As it was thought probable that some of the F_4 lines would resemble the Garnet parent by being resistant to stripe rust, the writer tested in the greenhouse the resistance of these lines to two physiologic races of stripe rust. Any lines that were resistant to all three rusts would be of considerable value as breeding material, in case Chinese wheats are susceptible to this rust.

The task of producing rust resistant wheats is complicated by the existence of many physiologic races of each of the cereal rusts. Little is known, as yet, about the specialization of these rusts in China. Tu (10), however, has shown that several physiologic races of stem rust of wheat are present, among which are included some races known to occur in America. In the present work, physiologic races collected in Canada were used for testing the Chinese varieties. Although such tests with rust of foreign origin may be of value in indicating which varieties possess rust resistance, a decision as to the suitability of the varieties for plant breeding purposes in China must depend on their resistance to rust races of Chinese origin.

As far as it is possible to judge from tests with rusts that are not of Chinese origin, it would appear that Chinese wheats are highly susceptible to stem rust but possess considerable resistance to leaf rust. It follows that, if Chinese varieties resistant to both these rusts are to be developed, the resistance to stem rust may have to be introduced from foreign varieties. It is possible, though by no means certain, that the so-called "mature plant resistance", which forms the basis of resistance to stem rust in most of the new American and Canadian spring wheats, may also be of value as a basis for resistance to rust in the spring wheat areas of China. The chief value of this type of resistance lies in the fact that varieties possessing it acquire, as they grow towards maturity, a resistance to all physiologic races, irrespective of how susceptible in the seedling stage they may be to the same races. If mature plant resistance should prove equally effective in China as in North America, it should not be difficult, by means of crosses, to combine it with the leaf rust resistance of Chinese wheats and other desirable qualities possessed by them.

Although nothing is known to the writer concerning the reaction of Chinese wheats to stripe rust, it appears that the course of action outlined above is also applicable to the production of varieties resistant to this rust. If the Chinese varieties should prove resistant to stripe rust, it will not be necessary to seek resistance to this rust from some other source. If they should prove susceptible, resistance to this rust must be sought elsewhere. It was with this possibility in view that the writer attempted to investigate the stripe rust resistance of Canadian spring wheats that already had the merit of resistance to stem rust and leaf rust.

Acknowledgments

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VEGETATIVE PROPAGATION OF CONIFERS

XI. EFFECTS OF TYPE OF CUTTING ON THE ROOTING OF NORWAY SPRUCE CUTTINGS[1]

By J. L. Farrar[2] and N. H. Grace[3]

Abstract

Full length Norway spruce cuttings, with and without a heel of old wood, were collected from the lower part of the tree at semimonthly intervals from July to October and were propagated in several media in outside frames.

Plain cuttings generally rooted better than cuttings with a heel of old wood, as judged by percentage rooted and the number and lengths of root. Heels, however, for summer collections favoured survival and rooting in sand, and root length in sand–peat. In several experiments involving early spring collections propagated in sand, the presence or absence of heels had little effect on the responses of the cuttings.

A late October collection involved six types of plain cuttings taken from the lower part of the tree and propagated in two different sand–peat media. In sedge peat medium there was little difference in the rooting of second order terminal, second order large lateral, second order small lateral, or third order lateral cuttings, the average rooting being 90%. Third order lateral cuttings showed an inferior development of new growth. The percentage of first order terminal cuttings rooted was 67%; these had markedly superior new growth development; when shortened, only 32% of such cuttings rooted. Irregular differences between the types of cutting occurred in the inferior sphagnum peat medium.

An important consideration in the vegetative propagation of conifers is the type of cutting used. Rooting and other responses are influenced by the age of the parent tree, the limb from which the cutting is taken, the part of the branch used to make the cutting, and its vigour (1–4, 6, 7, 9–14). This communication presents information on the reactions of cuttings made from twigs of different branch orders, cuttings with and without two-year old wood, and cuttings including all and part of the current year's growth.

Experimental

The experimental study of the effect of type of cutting on the rooting of Norway spruce involved seven experiments with 8500 cuttings all planted in outdoor frames. The experiments were of factorial design and the results pertaining to chemical treatments, propagation medium, and season of collection have already been presented (5, 8). Those data referred only to cuttings without old wood made from full length twigs 5 to 10 cm. long unless otherwise specified. The experiments designated in this paper were assigned the same number in a previous paper (5). Methods of collecting and planting the cuttings have already been described (8).

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Experiment 1

Full length cuttings 5 to 10 cm. long from the lower part of the tree with and without a heel of old wood were collected at semimonthly intervals during July and August, 1939, and planted in sand and sand mixed separately with sphagnum and sedge peats (5).

Experiment 2

Semimonthly collections were continued through September and October but were planted in sand and sand mixed with sedge peat only.

Experiment 3

Cuttings were collected from the lower part of the tree and were separated into five types as shown in Fig. 1. These five types of cuttings were all full length and plain, without a heel of old wood. Type 1 refers to first order terminals with an average length of 127 mm.; Type 2, second order terminals, 81 mm.; Type 3, second order large laterals, 93 mm.; Type 4, second order small laterals, 71 mm.; and Type 5, third order laterals, 59 mm. Cuttings of Type 6 are the distal part of Type 1 cuttings and had an average length of 79 mm.

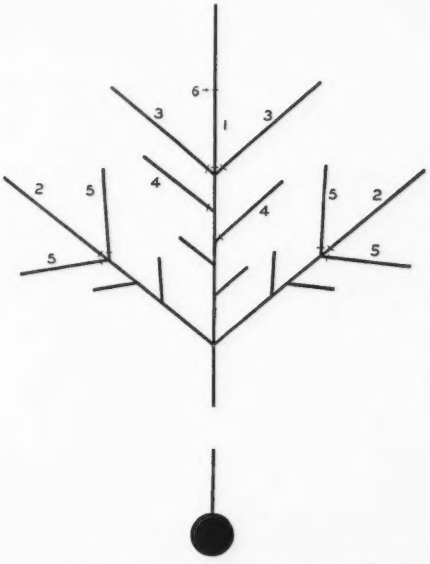

FIG. 1. *Diagrammatic representation of position on a lower branch of a Norway spruce tree from which cuttings of current season's growth were taken. Lines across twigs indicate position of cut. Type 6 is the distal part of a Type 1 cutting.*

In several experiments cuttings were planted in sand in outdoor frames before, during, and after initiation of new growth in the spring of 1939. Terminal and lateral cuttings were taken from the upper and lower part of

the tree; cuttings were both plain and with a heel of old wood, and some plantings involved full length lateral cuttings with both 1937 and 1938 growth. In 1938, some experiments involved cuttings made only from the lower portion of long twigs.

As indicated in a previous article (5), it has been possible to develop certain additional criteria on the greenhouse propagation in sand of long and short Norway spruce cuttings, plain and with heels. These data were recorded by the senior author at New Haven, Conn., in 1938, and the data subsequently published dealt chiefly with rooting (2, 4).

Cuttings of Experiments 1, 2, and 3 were removed for counts and measurements in September, 1940. Details of the observations made have been given in an earlier article (5). The cuttings planted in sand in the spring of 1939 were removed June, 1940, and counts were made of the number of cuttings surviving, rooted, and with new growth; the number and lengths of root were also determined. The cuttings planted in 1938 were examined in 1939. Statistical treatment of data from the responses of cuttings has been described previously (4, 6–8).

Results

The responses of the different types of cutting of Experiments 1, 2, and 3 are given in Table I. The data for Experiments 1 and 2 are averaged over four collections and four treatments as there were no important interactions between these factors and type of cutting. Data are given for responses in the three main types of media since there were marked interactions between the type of cutting and the propagation medium.

In Experiment 2 plain cuttings, on the average, were superior to those with a heel of old wood with regard to numbers and lengths of roots, and percentage rooted. These differences are illustrated in Fig. 2.

In Experiment 1, heels appeared to favour root length in the peat media, and survival and rooting percentage in sand; otherwise, plain cuttings were equal or superior.

In both Experiments 1 and 2, an almost equally high percentage of each type of cutting rooted in the favourable sedge peat medium.

Sand propagation in the greenhouse at New Haven, Conn., demonstrated that long cuttings had 3.8 roots with a length of 104 mm. per rooted cutting while short cuttings had 3.0 roots and a length of 63 mm. There was no significant difference between plain cuttings and those with a heel in respect to either number or lengths of roots per rooted cutting. Mortality of the long cuttings was substantially greater than that of the short, and was unaffected by heels. This increased mortality is of interest since the long cuttings rooted better than the short (2, 4). Long cuttings developed new growth to a greater extent than short, and the plain were superior to those with heels.

In the experiments done in outdoor frames in sand in the spring of 1939, the presence of heels had little effect, though heels resulted in a slight increase in the mean root length. Of the cuttings with the full length of two years'

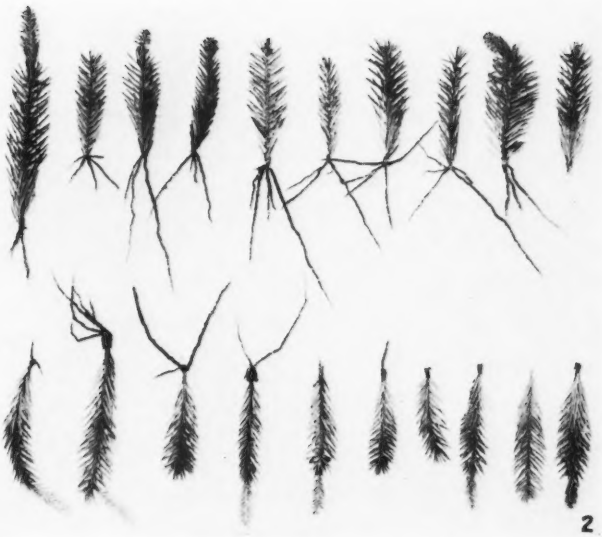

FIG. 2. *Norway spruce cuttings propagated in sand. Upper: plain cuttings without a heel of old wood. Lower: cuttings with heel of old wood.*

FIG. 3. *Norway spruce cuttings propagated in sand–sedge-peat. Upper: shortened first order twigs (Fig. 1, Type 6). Lower: full length first order twigs (Fig. 1, Type 1).*

TABLE I

EFFECTS OF TYPE OF CUTTING ON RESPONSES OF NORWAY SPRUCE AND INTERACTION EFFECTS WITH MEDIA

Criteria	Medium	Experiment 1		Experiment 2		Experiment 3					
		Cuttings		Cuttings		Type of cuttings†					
		Plain	With heels	Plain	With heels	1	2	3	4	5	6
Number of cuttings surviving, %	Sand	22	53	92	97						
	Sphagnum peat	78	80			30	62	77	73	47	73
	Sedge peat	98	98	98	98	87	92	93	97	93	53
Number of cuttings rooted, %	Sand	14	20	80	39						
	Sphagnum peat	58	38			13	35	47	47	30	22
	Sedge peat	92	88	95	83	67	88	87	95	90	32
Number of cuttings rooted as percentage of number surviving	Sand	61	37	87	40						
	Sphagnum peat	75	42			44	57	61	64	64	30
	Sedge peat	93	90	97	84	77	96	93	98	96	59
Number of roots per rooted cutting	Sand	3.5	2.9	4.0	2.5						
	Sphagnum peat	3.7	3.7			2.4	4.1	2.8	2.9	3.6	1.8
	Sedge peat	4.7	4.5	4.6	3.0	4.6	4.2	4.5	4.2	3.8	2.7
Length of roots per rooted cutting, mm.	Sand	91	65	107	56						
	Sphagnum peat	149	157			79	175	115	98	137	71
	Sedge peat	220	261	234	133	255	230	277	203	192	89
Mean root lengths, mm.	Sand	26	23	26	22						
	Sphagnum peat	40	43			33	43	41	33	38	38
	Sedge peat	47	58	51	44	56	55	61	48	50	33
Number of cuttings with new growth, %	Sand	4	2	45	42						
	Sphagnum peat	2	2			18	17	15	10	7	23
	Sedge peat	54	46	67	60	87	75	87	72	48	50
Number of cuttings with new growth as a percentage of those surviving	Sand	8	2	49	43						
	Sphagnum peat	3	2			61	27	20	14	14	32
	Sedge peat	54	47	68	62	100	82	93	74	52	94
Number of rooted cuttings with new growth as a percentage of those rooted	Sand	7	3	50	50						
	Sphagnum peat	4	3			50	38	29	18	11	38
	Sedge peat	55	49	69	63	100	81	92	74	52	95
Number of rooted cuttings with new growth as a percentage of those with new growth	Sand	X	X	89	46						
	Sphagnum peat	X	X			36	80	89	83	50	36
	Sedge peat	95	94	98	86	77	96	92	98	97	60
Number of shoots per cutting with new shoots	Sand	X	X	1.2	1.1						
	Sphagnum peat	X	X			1.3	1.3	1.0	1.0	1.0	1.0
	Sedge peat	1.4	1.4	1.4	1.2	3.1	1.4	1.6	1.2	1.1	1.7
Mean length of longest shoot on each cutting with new shoots, mm.	Sand	X	X	19	17						
	Sphagnum peat	X	X			26	16	26	15	11	17
	Sedge peat	21	21	28	23	41	35	41	29	34	33

† *See Fig. 1.*
X = *Meagre data.*

growth, 91% survived the test period, but only 30% were rooted; survival of full length plain cuttings of one year's growth was 89%, with 41% rooting.

It is apparent from the data of Experiment 3 that there were only small differences between the rooting responses of Types 2, 3, 4, and 5 when propagated in a sedge peat medium, though third order lateral cuttings (Type 5) were rather poor in respect to development of new growth. Somewhat greater differences occurred among these types when planted in sphagnum peat. The first order terminal cuttings, Type 1, were definitely below Types 2, 3, 4, and 5 in respect to percentage rooted, were quite equal to them in respect to numbers and lengths of roots, and superior in respect to new growth. When shortened to a length approximately equal to Types 2, 3, and 4, such first order terminal cuttings (Type 6) were much inferior to the other types, especially when planted in the superior sedge peat medium. It might be noted that practically all surviving cuttings of Types 1 and 6 bore new growth. However, for all classes of cuttings, those surviving rooted equally well whether they bore new growth or not. Furthermore, comparison of percentage rooted and percentage with new growth shows that a type of cutting favouring one condition does not necessarily favour the other. The effects of shortening first order terminal cuttings are illustrated in Fig. 3, which shows the living cuttings from representative groups of 10 cuttings.

Spring plantings in sand confirmed earlier results on the effect of position of the cutting on the tree and on the branch (6, 13). Cuttings taken from the lower part of the tree rooted better than those from the upper region and lateral cuttings were consistently superior to the terminals.

Some cuttings made from the lower part of the twig, that is, without a terminal bud, and planted in a mixture of sand and sphagnum peat in 1938 did root, though the percentage was small. In this connection it may be noted that cuttings in which the new growth all died (8) were not prevented from rooting. This indicates that the terminal bud is not essential to the rooting of cuttings.

Discussion

The results suggest that it is important that cuttings be made the full length of the current year's growth. Though short cuttings might be superior to long, the latter were not improved by shortening them. There was no advantage in making cuttings with a heel.

The position of the cutting on the branch had little effect if the cuttings were made from twigs about 6 to 9 cm. long and planted in the sedge peat medium. Long terminal cuttings also rooted in fair percentages in the same medium. Since these larger cuttings show good development of roots and new growth, their propagation may be of value in overcoming the plagiotropic growth habit evidenced by spruce cuttings.

The greater development of new growth in sedge peat medium suggests that substances absorbed by the cutting affect bud development. The reduction in new growth when cuttings are shortened may possibly be related to the nutrients available in the twig.

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THE EFFECT OF ETHYL ALCOHOL AND TRICRESOL ON THE VITAMIN A CONTENT OF THE BLOOD AND LIVER OF THE CHICKEN[1]

W. A. MAW[2] AND N. NIKOLAICZUK[3]

Abstract

That preservatives in perishable biological extracts may specifically alter physiological function cannot be precluded in the analysis of experimental results. Whereas 15% ethyl alcohol is shown to significantly lower both vitamin A and carotene concentrations of the liver in growing chickens, little effect is manifest upon similar blood constituents; 0.1% and 1.0% levels of tricresol were without effect upon either vitamin A or carotene in blood or liver, but prompted a pronounced increase in total mean body weight of 30% above the control; alcohol slightly depressed gains. Both preservative materials under study markedly reduced the amount of carotenoids in the shank skins. The extent of such effects in herein reported.

Introduction

The storage of biological extracts for future use necessitates the addition of preservatives such as dilute ethyl alcohol and tricresol. Ethyl alcohol when added to yield a concentration of 15% and tricresol at a level of 0.1% have been found to possess excellent preservative qualities. The effect of these admixtures and their relation to the problem under study cannot be disregarded in the analyses of the results.

Studies at Macdonald College (2, 4) demonstrated that the metabolic fraction of the anterior pituitary (622*c*), in which 15% ethyl alcohol was used as a preservative, gave an increased vitamin A content in the liver of the growing chicken. It was observed also that alcohol tended to decrease this effect. These findings were further substantiated in later work (3) in which the carotenoid contents of the rations were under study.

Data published by Clausen (1) suggest an effect of ethyl alcohol on vitamin A metabolism in dogs and the authors' experiments prove a similar effect upon chickens.

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Contribution from the Faculty of Agriculture of McGill University, Macdonald College, Q. e., Canada, with assistance from the National Research Council, Ottawa, Canada. Macdonald College Journal Series No. 164.

[2] *Assistant Professor of Poultry Husbandry and Head of the Department of Poultry Husbandry.*

[3] *Lecturer, Department of Poultry Husbandry.*

Experimental

Forty Leghorn cockerels, 10 weeks in age, were randomized for body weight and allotted to four groups of 10 birds each. All lots were fed a dry mash and water ad libitum. Lot 1, the control group, was injected intramuscularly with $\frac{1}{2}$ cc. distilled water twice daily. Lots 2, 3, and 4 received similar injections of 15% ethyl alcohol, 0.1% and 1.0% tricresol, respectively.

The period of treatment was 21 days, after which all birds were killed and blood samples were taken by severing the jugular vein posterior to the angle of the lower mandible. The blood was allowed to clot and the serum was collected for vitamin A and carotene analyses. The livers were removed for immediate analyses after the birds were bled. The shank skins were also analysed for carotenoid pigment content.

Results

Data presented in Table I reveal that ethyl alcohol has a marked influence on the amount of vitamin A in the liver and the carotene of both the liver and shank skins, as shown by the appreciably lower values for these constituents when compared with both control and tricresol lots. The blood carotene and vitamin A do not appear to be significantly affected by the alcohol, but some reduction in the former is observed. The carotene and vitamin A contents of the liver are significantly lower than those of the control group, which would suggest that mechanisms of withdrawal are stimulated by the continuous presence of alcohol or its effect in conjunction with normal metabolic processes.

TABLE I

THE EFFECT OF ETHYL ALCOHOL AND TRICRESOL UPON THE MOBILIZATION OF VITAMIN A POTENT SUBSTANCES IN THE LIVER AND BLOOD OF GROWING CHICKENS

(Summary of 10 individuals per lot)

Treatment*	Gain in body weight, gm.	Liver weight, gm.	Percentage of total body weight	Liver carotene, mg./gm.	Liver vitamin A, mg./gm.	Blood carotene, mg./100 cc. serum	Blood vitamin A, mg./100 cc. serum	Shank skin carotenoids, mg./gm.
Water	296	18.29	1.77	1.03	15.80	0.725	0.072	0.027
Ethyl alcohol	277	18.55	1.95	0.55	11.80	0.682	0.077	0.013
Tricresol, 0.1%	397	24.24	2.20	1.29	14.50	0.711	0.067	0.019
Tricresol, 1.0%	383	21.13	2.04	1.04	14.30	0.791	0.065	—

* *Injection intramuscular, $\frac{1}{2}$ cc. twice daily.*

Tricresol at 0.1% and 1.0% levels has no marked effect upon the vitamin A and carotene content of the liver, nor upon the quantity of these constituents in the blood serum.

Whereas alcohol depressed growth as measured by mean gain in body weight, 277 gm. as compared with 296 gm. for the control, tricresol at both

levels prompted greater total gains, approximately 30% higher than the control. The specific significance of the growth promoting properties of tricresol is obscure on the basis of the data recorded in this experiment.

Liver weights in the tricresol groups are significantly greater than in the control group. This is, undoubtedly, the effect of tricresol treatment as demonstrated by the increased proportion of liver weight to body weight, namely, 2.20% and 2.04% as compared with 1.77% for the control and 1.95% for the alcohol group.

The carotenoid content of the shank skins is greatly reduced by alcohol and tricresol injections.

Acknowledgments

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A NEW SPECIES OF *ONCHIDIOPSIS* FROM BAFFIN LAND

By Henry D. Russell

Abstract

A detailed description of a new species of gastropod mollusc, *Onchidiopsis kingmaruensis*, is given. The specimen on which the description is based is the second of this genus to have been found in the western Atlantic region and was collected at Lake Harbour Fiord, Baffin Land, by Dr. John Oughton of the Royal Ontario Museum, Toronto, Ont. The species is compared with *O. corys* Balch.

While on a trip to Labrador and Baffin Land during 1939, Dr. John Oughton of the Royal Ontario Museum, Toronto, Ont., collected a single specimen of *Onchidiopsis* which he has most kindly sent to the author for identification. The specimen was obtained on August 4 in 30 fathoms of water at Lake Harbour Fiord, Baffin Land. After a critical examination the author believes the specimen to be a new and valid species; a description and comparison with *O. corys* Balch follows below.

Onchidiopsis kingmaruensis n. sp.*

(Figs. 1 to 9)

The holotype is No. 17,260 in the collection of the Royal Ontario Museum of Zoology, Toronto, Ont.

Size

The length of the specimen preserved in alcohol is 24 mm.; the width, 15 mm.; and the height, 17 mm.

Colour

Foot, head, and notaeum are uniformly grayish-yellow, suggestive of a water blister; eye spots black.

General Form (Fig. 4)

The notaeum is wrinkled over its entire surface suggesting the contours of the human brain. These wrinkles become more pronounced as they near the ventral border. The notaeum rises at an angle of 25° from the anterior towards the posterior end and covers the large visceral hump.

Below the notaeum the foot of moderate size bearing dorsolaterally ruffled edges is plainly visible. Its blunt anterior angles and notched anterior edge extend slightly beyond the origin of the tentacles. Posteriorly, it terminates in a blunt point just before reaching the posterior edge of the notaeum. It turns sharply upward at its posterior extremity for about one-fourth its length as described by Balch (1, p. 475).

[1] *Manuscript received September 18, 1941.*
Contribution from the Museum of Comparative Zoology, Cambridge, Mass., U.S.A.

[2] *Assistant Curator of Molluscs.*

* *The name "Kingmaru" is the Eskimo word for Lake Harbour.*

The thick obtusely pointed tentacles extend for half their length beyond the anterior edge of the foot. Laterally the circular black eyes are borne at a point about halfway between the origin and tip of these tentacles.

The large penis situated on the right side of the neck and dorsal to the right tentacle is not visible beneath the anterior portion of the notaeum.

Notaeum

The notaeum is fleshy and deeply wrinkled except over the dorsal part of the visceral mass where the wrinkles become less well defined. Slightly to the left of the median line anteriorly is the ill defined inspiratory cleft and on the right at a point two-fifths of the distance between the anterior and posterior extremities of the animal is located the far more distinct expiratory cleft. Both of these, however, resemble folds in the notaeum rather than actual clefts.

Shell (Fig. 3)

The shell is chitinous, very thin, and situated immediately beneath the notaeum. It appears as a transparent horny film. There is a small central point at the otherwise straight-edged anterior end and the posterior edge is rounded. The sculpture consists of concentric growth lines with the nucleus at the anterior end. The shell is highly arched both from the sides and ends towards the centre. It measures 19 mm. long, 10 mm. wide, and 6 mm. high at the centre, its highest point. It is somewhat firmer than that described by Balch (1, p. 471) since when held by forceps it will support itself against gravity in air. This is true only if it is held by the posterior end.

Gill cavity and branchial complex

On opening the arched branchial chamber situated on the dorsal side of the neck and between it and the visor-like anterior fold of the notaeum which covers the head, the osphradial and branchial laminae are immediately visible. As the observer holds the animal in the normal position for crawling, with the notaeum uppermost, the head facing him and the foot below, the gill appears on the left and the osphradium on the right of the cavity. The gill (Fig. 5) consists of 22 simply pointed, cream coloured laminae that extend into the branchial chamber from its thin dorsal wall. The osphradium consists of a double series of 11 leaflets supported by a central rachis and extending into the branchial chamber from the dorsal wall. The leaflets are unequal in shape, those on the left being larger and more pointed than the semicircular ones situated on the right side of the rachis. Both sets of leaflets possess a black pigment colouring their dorsal edge. Except for this black pigment the osphradium is cream coloured and the rachis is white.

Tentacles and eyes

The tentacles arise from the anterior of the dorsoventrally sloping head. They are bluntly pointed and about half as wide as long. For half their length they are faintly wrinkled and uniform in width. Then they swell slightly and become more deeply wrinkled and gradually narrow to a blunt

point. The eyes (Fig. 8) are small, round, black, and laterally situated on the tentacles at the point where the swelling takes place.

Foot (Figs. 5, 8, 9)

The foot in contraction is about three times as long as wide. Anteriorly it terminates in short, blunt, lateral angles or auricles. The anterior edge is slightly notched at the centre and possesses a deep horizontal groove which extends from the tip of one auricle to the tip of the other. Posteriorly the foot terminates in a bluntly rounded point which turns sharply upward against the notaeum. On the dorsal surface of the foot and extending posteriorly from the base of the anterior angles is a distinct, irregularly ruffled area 2 mm. wide. It is slightly narrower at its anterior and posterior ends than at the central portion. This ruffle is composed of dorsoventral folds that are somewhat higher than the dorsal surface of the foot (Fig. 9). Where the posterior dorsal surface between the lateral ruffles presses against the notaeum it possesses a triangular thickening or pad. The plantar surface of the foot is cream coloured, but the dorsal surface is tinged with gray.

Radula

The radula (Fig. 1) is taenioglossate and has the formula

$$\frac{1}{2}, \frac{5+1+5}{1}, \frac{5+2+1+1+5}{1}, \frac{5+1+5}{1}, \frac{1}{2}.$$

The uncinal hooks (Fig. 1A) are long, non-dentate, smooth, and sharp. The claw-like part of the older teeth is about as long as the base. That of younger teeth is about twice as long as the base and is longer, narrower, and more sharply pointed than is that of the older teeth (Fig. 2).

The elliptical reflection of the laterals (Fig. 1B) bears four to five small bluntly pointed cusps on either side of the long sharply pointed median cusp. The base of the laterals is bluntly rounded, more slender and triangular in shape than is that of the median teeth.

The older median teeth are mushroom-shaped with a very broad and short "stipe". The elliptical reflection is flat across its anterior surface or slightly depressed towards the centre. It bears five to six small bluntly pointed cusps on either side of the much longer and more sharply pointed central one. The sides of the pillar or "stipe" are concave and enlarge into an obtusely rounded basal portion. The younger medians (Fig. 1C) bear two or three small sharply pointed denticles at the base of and lateral to the large central cusp. Usually there is one small cusp on one side and two on the other, making a total of 13 to 14 cusps. The presence of these small cusps on the younger teeth and the lack of them on the older ones seems to be the only difference between the medians of varying ages in the radula.

Other Characters

The mouth appears merely as a horizontal slit between and slightly ventral to the tentacles. No rostrum was observed.

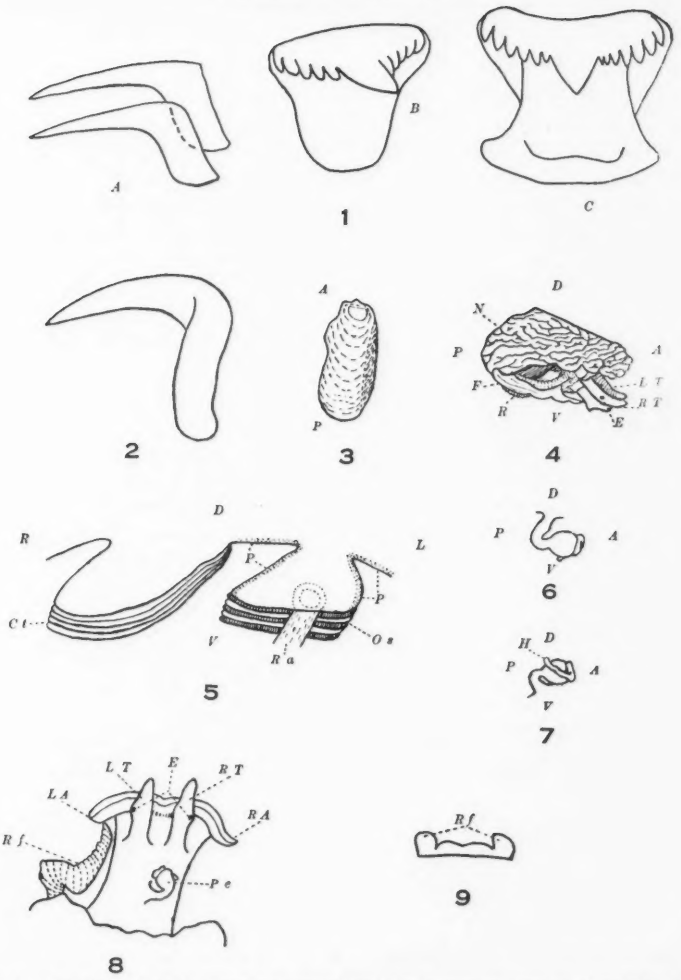

FIG. 1. *Left half of single transverse row of teeth from younger portion of radula. A, uncinal teeth; B, lateral tooth; C, median tooth (greatly enlarged).*

FIG. 2. *Uncinal tooth from older portion of radula (greatly enlarged).*

FIG. 3. *Dorsal aspect of shell. A, anterior; P, posterior. Natural size.*

FIG. 4. *Right lateral view of animal. A, anterior, P, posterior end; D, dorsal, V, ventral surface; N, notaeum; F, foot; R, ruffle, LT, left tentacle; RT, right tentacle; E, eye. Natural size.*

FIG. 5. *Branchial complex. R, right side; L, left side; D, dorsal, V, ventral surface; P, pigmented area; Ra, rachis of osphradium; Os, osphradium; Ct, ctenidium (greatly enlarged).*

FIG. 6. *Penis. A, anterior, P, posterior end; D, dorsal, V, ventral rod of penis (greatly enlarged).*

FIG. 7. *View of under side of penis showing the rod, H; D, dorsal, V, ventral surface; A, anterior, P, posterior end (greatly enlarged).*

FIG. 8. *Dorsal aspect of head. RA, right anterior angle of foot; LA, left anterior angle of foot; RT, right tentacle; LT, left tentacle; E, eyes; Rf, ruffle of foot; Pe, penis. ×3.*

FIG. 9. *Transverse section through posterior portion of foot, showing dorsolateral ruffles, Rf. ×4.*

The fragile jaws were not examined, probably having been destroyed during the removal of the buccal mass. It is probable, however, that they are similar to those described by Bergh (2, Pl. 3, Fig. 29, *a* to *b*) and by Balch (1, Pl. 22, Fig. 4).

About midway between the right tentacle and the branchial chamber is the large penis (Figs. 6, 7, 8). This arises slightly to the left and dorsal to the right tentacle. As it proceeds laterally, it narrows and bends almost directly back upon itself swelling suddenly to about twice its original width and resembling a clenched fist. This is capped by a single rounded coil of tissue which after making one complete turn continues as a rod along the side of the swollen portion of the penis and between it and the dorsolateral wall of the neck (Fig. 7H). The penis is somewhat wrinkled over its entire surface.

In Table I, the characters described above are summarized and compared with those of *O. corys*.

TABLE I

COMPARISON OF THE CHARACTERS OF *O. corys* BALCH AND *O. kingmaruensis* RUSSELL

Characters	*O. corys*	*O. kingmaruensis*
1. Colour	In formol:— ground colour purplish-brown with darker markings on the lower sides; colour pattern gives the effect of 12 ill defined blotches of the ground colour.	In alcohol:— uniformly yellowish-gray; no colour pattern.
2. Notaeum	Smooth on top and sides, wrinkled or strongly folded and vesiculate elsewhere.	Wrinkled over its entire surface, but much more strongly so on the sides than immediately over the visceral mass.
3. Shell	Very thin; does not resist the action of gravity in air; posterior–inferior portion folds into the posterior–superior portion.	Thin; can resist the action of gravity in air as above mentioned; shell more elongate than that of *O. corys;* no infolding of the posterior–inferior portion as in *O. corys*.
4. Branchial complex	Osphradium consists of a double row of bilobed leaflets, approximately equal in shape.	Osphradium consists of a double row of leaflets unequal in shape as above related.
5. Foot	Posterior; projects beyond the notaeum; dorsolateral edge smooth.	Posterior; does not project beyond the notaeum; dorsolateral edge deeply ruffled.
6. Radula	Median tooth with 6 to 10 lateral denticles; laterals with a median cusp flanked by seven denticles on the external and five on the internal side; uncini with smoothly arched claw.	Older median tooth with five to eight lateral denticles on each side; younger median with eight to nine lateral denticles on each side; laterals with four to five denticulations on either side of the median cusp; uncini with smooth arched claw.

Discussion

Of the four known species and subspecies of *Onchidiopsis* given by Balch (1, p. 477) only one, *O. corys* Balch is neoboreal or western Atlantic in distribution. Thus *O. kingmaruensis* is the second specimen and species of this genus to be reported from this side of the Atlantic, since Balch's specimen was a unique. There are so many character differences between *O. corys* and *O. kingmaruensis* that the author feels no hesitancy in believing this latter to be a new species.

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